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COMPARISON OF APHID AND MANUAL METHODS FOR TRANSMITTING INTERNAL CORK AND ASSOCIATED VIRUSES OF THE SWEETPOTATO

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SUMMARY

The study compared aphid and manual inoculation of sweetpotato with viruses associated with internal cork. All aphid inoculations were with *Myzus persicae*.

Two seedlings, La. II and La. IX, proved much more susceptible to internal cork than the standard (Porto Rico), with respective infection of 80, 75, and 27%, and severity scores of 1.50, 2.40, and 0.84 for the manual inoculation, compared with 30, 25, and 10% infection and 1.25, 0.95, and 0.38 for the aphid method. Three sources of internal cork inocula behaved similarly in all years.

On most of the other sweetpotato seedlings the two methods of inoculation were about equally effective on the average, but there were more zero values for root lesions in the aphid-inoculated than in the manually inoculated plants throughout this 3-year study.

When the initial amount of inoculum per inoculated plant was increased, the two methods of inoculation remained about equally effective. Secondary spread of the virus from inoculated plants into uninoculated check plants was noted in plantings inoculated by both methods. When the proportion of uninoculated checks was increased to 80% of the plants in the field secondary spread was observed only in the field of manually inoculated plants.

The feathery mottle syndrome was shown to involve three different viruses.

The name internal cork is derived from the appearance of necrotic lesions that occur in sweetpotato roots in storage, chiefly above 60°F. In some earlier studies at Beltsville, an incubation period of 6 months in warm, humid storage (75°, 85%) was required for single inoculations (aphid and graft) to produce the smallest (trace) root lesions in the Porto Rico variety grown in the greenhouse (6, 7). Since only 20% of the inoculated plants produced roots with lesions, compared with 100% having foliage lesions, it was possible that under these conditions the cork virus was but a component of a mixture in which a leafspot virus predominated. It had already been found that the internal cork virus was involved in feathery mottle (4). Consequently, a study was begun in early 1957 in which the two proved aphid and manual (1) methods of inoculation were compared for transmission of the internal cork virus complex and its development under field conditions.

Materials and methods.—All the plants to be inoculated were virus-free unless otherwise specified.

In the early stages of the work, efforts were made to find virus-free sweetpotato clones that might differentiate the internal cork virus (or viruses) and other viruses associated with it, better than those previously used.

To that end, clones of numerous seedlings were inoculated and grown in the greenhouse and symptoms noted monthly for 3 months. Some hundreds of virus-free seedlings were grown from open-pollinated seed of Porto Rico collected in 1956 in Louisiana. In 1957, clones of 57 of these, along with two checks, were grafted, in duplicate, with tip scions from plants exhibiting the internal cork and feathery mottle virus syndromes. Seven of these clones (La. I, II, III, VI, VII, IX, XXI) were selected along with two others, B-6360, and HM-15, because of their relatively high sensitivity and marked symptom expression, for determining whether any of them might be superior to Porto Rico as indicators of the root-lesion phase of internal cork. Concurrently with their evaluation as indicators these seedling clones and Porto Rico served as the test materials for the inoculation studies.

For the field growth tests, except for row 7 in field M in 1957, all plants were inoculated under controlled conditions in the greenhouse: 1) by placing 15-20 green peach aphids (*Myzus persicae* (Sulz.) on a single leaf of each plant employing the nonpersistent-virus-transmission technique (7); and 2) by the manual squeeze-rub-rapid-transmission technique on a single leaf. The inoculated plants were transplanted to 2 similar, but widely separated, field plantings designated as A (for aphid inoculation) and M (for manual inoculation). Each planting was 7 rows of 50 plants each, spaced 2 ft. apart in rows 4 ft. apart. All plants were inoculated.

Three sources of inoculum were used in 1957 to determine their relative infectivity: 1) Porto Rico leaves showing mainly feather (f) and oak-leaf (ol) patterns, 2) Scarlett O'Hara morning glory foliage containing virus isolated from sweetpotato root lesions, and 3) diseased Porto Rico foliage showing mainly chlorotic spots (cs). All three sources were used on Porto Rico, La. I, La. VII, and La. XXI; two sources were used on La. II and La. IX, and one source on the remainder. A week after transplanting, row 7 in field M (of virus-free La. I) was grafted with chip-buds of material infected with feathery mottle.

The arrangement and sources of scions in row 7 were as follows: In field M, five 10-plant increments of the row were grafted with buds from 5 plant sources, respectively: 1) Russian variety (P.I. 105945); 2) Georgia Red variety from H. W. Rankin, of Georgia; 3) La. XXII inoculated earlier from Russian; 4) Georgia Red from S. A. Harmon; and 5) La. X inoculated earlier from Russian. In field A four increments of row were set, respectively, with plants that had been inoculated by aphids in the greenhouse with inoculum from the first four sources just enumer-

ated: 1) plants 1-13; 2) plants 14-26; 3) plants 27-30; and 4) plants 31-50. Plants 1-10 were transplanted on June 5; 11-30 on June 12; and 31-50 on June 19.

Adverse cool weather, which followed planting on May 16, 1957, injured plants in the field and made it necessary to repeat many of the manual inoculations on new plants on June 6 and plant them as replacements in the field. The cool period in late May and a dry period in August were departures from the typical pattern of the growing season.

In the M field, reinoculations were made on July 5 on the first 5 plants in each series of 10 in rows 1-5, and again on July 30 on the first 3 plants in each series.

In 1958, seedlings La. I, La. VII, and La. XXI, and the Porto Rico variety were planted in 7 rows in the two fields A and M after inoculation by the same techniques as in 1957. In each row of 50 plants, plants 11-20 and 31-40 were inoculated with sweetpotato root lesion inoculum from Scarlett O'Hara morning glory, and the others served as uninoculated checks. More than 50 viruliferous aphids were placed on 3-5 leaves per plant instead of less than 20 per plant on a single leaf, as in 1957.

In 1959, procedures were similar to those used in 1958, but only plants 21-30 in each row were inoculated. Seedlings La. I and La. IX and Porto Rico variety were inoculated as described for 1958. One row (row 4) of a second apparently virus-free stock of Porto Rico was inoculated with leafspot virus only.

Each year before inoculation of the test plants, the inoculum sources were indexed on Scarlett O'Hara morning glory for virus presence and held in the greenhouse for future use.

Also in 1959, 15 plants each of La. I, La. VII, La. IX, B-6360, Nemagold, Yellow Jersey, and Porto Rico were inoculated with internal cork inoculum by both methods and grown in the greenhouse for 3½ months.

After the harvest and curing (85° for 10 days) were completed, the roots were placed in warm storage (75°, 85% RH) for 7½ months, and then were sliced for recording incidence and severity of root lesions. Four classes of injury were given arbitrary numerical values, approximately proportional to the degrees of severity of impairment of the sweetpotato root as food, and a severity index was computed for each lot (5).

Results and discussion.—The results from one variety and 3 seedling clones inoculated with three sources of inoculum in 1957 are summarized in Table 1. A large proportion of the plants developed foliage symptoms and produced roots of commercial size. Root lesions were produced by varying numbers of inoculated plants.

The two methods of inoculation were about equally effective, on the average, with the inocula and on the varieties shown in Table 1. However, there were more zero values for root lesions in the aphid-inoculated than in the manually-inoculated plots.

Table 2 shows the results of similar inoculations of 5 or 10 plants each with only one or two of the three

Table 1.—Comparison of aphid (A) and manual (M) inoculation on 4 sweetpotato clones with internal cork inocula from 3 sources (1957).[a]

Row	Clone and method of inoculation	No. of plants inoculated	% plants with roots	% plants with leafspot	% plants with root lesions from indicated inocula[b] 1	2	3	Total	Severity score (5)
1	Porto Rico								
	M	50	82[c]	86	10	35	15	27	.84
	A	50	84	60	0	15	5	10	.38
2	La. VII								
	M	50	83	94	10	10	10	11	.20
	A	50	86	78	20	10	0	9	.16
3	La. I								
	M	50	96	96	20	20	0	13	.24
	A	50	92	78	40	30	5	24	.44
4	La. XXI								
	M	50	96	94	10	20	15	17	.32
	A	50	92	84	0	15	15	13	.24
Mean									
	M	200	90	93	13	21	10	17	.40
	A	200	91	75	15	18	6	14	.31
	Grand mean		90	84	14	19	8	15	

[a] In each pair of lines, the first line (M) shows the results of manual inoculations, and the second line (A) is for aphid-inoculations.

[b] The 3 inocula used were: 1) leaves showing feather and oak leaf from diseased Porto Rico on 10 plants, 2) Scarlett O'Hara morning glory foliage with virus isolated from sweetpotato root lesions on 20 plants, and 3) chlorotic spotted foliage (cs) from diseased Porto Rico on 20 plants.

[c] Plants or hills were not counted unless roots were available for slicing.

Table 2.—Comparison of aphid (A) and manual (M) methods of inoculation of sweetpotato clones with internal cork inocula from three sources (1957).

Row	Clone and inoculation method	No. of plants inoculated	% plants with		% plants with root lesions from indicated inocula				Severity score (5)
			roots	leafspot	1	2	3	Total	
5	La. II								
	M	20	50	50	67		100	80	1.50
	A	20	50	65	60		0	30	1.25
	La. IX								
	M	20	60	60		86	67	75	2.40
	A	20	80	80		50	0	25	.95
	La. VI								
	M	10	70	90		43		43	.60
	A	10	60	70		20		20	.20
6	Porto Rico								
	M	10	100	100	20			20	.40
	A	10	50	100	10			10	.20
	La. VII								
	M	10	80	70		50		50	1.60
	A	10	80	90		50		50	1.80
	La. XXI								
	M	10	30	70		0		0	.00
	A	10	70	80		0		0	.00
	La. III								
	M	5	60	60			100	100	1.20
	A	5	80	80			0	0	.00
	B-6360								
	M	5	100	100			25	25	.40
	A	5	80	100			40	40	.80
	HM-15								
	M	10	70	20			28	28	.40
	A	10	80	40			0	0	.00

inocula indicated. Most plants exhibited symptoms and in 6 of the 9 clones there was a consistent difference between the two methods of inoculation. Manual inoculation appeared better because aphid inoculation was followed by more zero values for root lesions and a lower mean for root-lesion occurrence.

Of the 50 chip-bud scions containing feathery mottle viruses, only 5 survived, but the stock plants grew and produced roots. The aphid-inoculated plants in row 7 survived and produced roots comparable to the chip-grafted ones. Eighty-four percent of the grafted plants and 82% of aphid-inoculated plants showed leaf spots; and 20% of the grafted plants showed internal cork root lesions, whereas none of the aphid-inoculated plants did. Of the five plants on which grafts survived, 2 showed yellow dwarf symptoms. Thus the results of grafts of feathery mottle inoculum agree with earlier findings that the feathery mottle complex involves three virus syndromes—leafspot, internal cork, and yellow dwarf (4).

Greenhouse-inoculated plants grown in the field produced roots with larger cork lesions than in earlier studies, in which greenhouse-grown plants produced rather poorly developed roots.

Among 65 plants reinoculated in the field, 36 had root lesions (55% infection). Among 65 comparable non-reinoculated plants, 29 had root lesions (45% infection).

The 1958 and 1959 results with three sources of cork inoculum are shown in Table 3. In both years the incidence of root lesions differed greatly among rows of a single variety or seedling within each field.

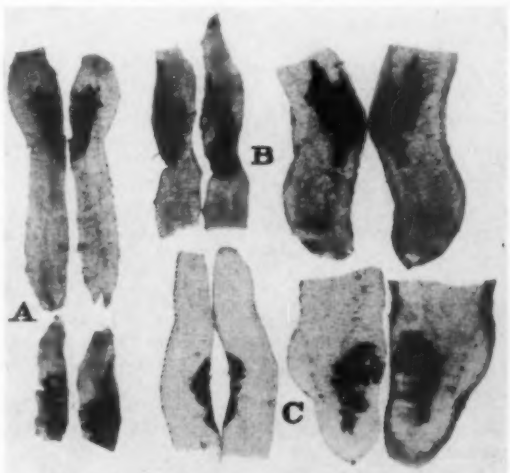

Fig. 1.—Lesions of internal cork in roots of sweetpotato seedlings grown in the field during 1957 and sliced after 7½ months' warm storage: A, La. II; B, La. IX; and C, B-6360.

Table 3.—Comparison of aphid (A) and manual (M) methods of inoculation with internal cork inoculum from Scarlett O'Hara morning glory on sweetpotato selections grown in the field at Beltsville in 1958-59.

Year and row	Clone and method of inoculation	% diseased with internal cork		Severity score (5)	
		Inoculated	Checks	Inoculated	Checks
1958 [a]					
1	La. I				
	M	20	3	.70	.13
	A	25	6	.50	.13
2	Porto Rico				
	M	10	3	.20	.07
	A	40	7	1.65	.23
3	Porto Rico				
	M	70	15	2.25	.73
	A	35	3	1.50	.07
4	La. VII				
	M	5	3	.10	.07
	A	0	0	.00	.00
5	La. XXI				
	M	20	3	.40	.07
	A				
6	La. I				
	M	30	3	.75	.07
	A	10	15	.20	.37
7	La. I				
	M	5	3	.10	.07
	A	30	20	.60	.40
1959 [b]					
1	Porto Rico				
	M	40	3	3.20	.05
	A	30	0	1.40	.00
2	La. I				
	M	40	0	1.60	.00
	A	40	0	1.10	.00
3	La. IX				
	M	20	0	.70	.00
	A	0	0	.00	.00
5	La. I				
	M	40	0	1.10	.00
	A	0	0	.00	.00
6	La. IX				
	M	60	0	1.80	.00
	A	10	0	.20	.00
7	Porto Rico				
	M	80	20	3.80	.10
	A	10	0	.20	.00
Mean [c]					
Mechanical		30	3	1.30	.10
Aphid		20	3	.61	.10

[a] Each row contained 20 inoculated and 30 check plants.
[b] Each row contained 10 inoculated and 40 check plants.
[c] Mean percentages are weighted means. Mean severity scores are nonweighted.

The two methods of inoculation were apparently as effective in 1958 as in 1957, but in 1959 the aphid method was much less effective.

In 1958, when the 2nd and 4th 10-plant increments of each row consisted of inoculated plants, the spread of virus to check plants was much higher than in 1959, when only the 3rd increment was inoculated.

Of the seven kinds of inoculated sweetpotatoes grown for 3½ months in the aphid-free greenhouse in 1958, La. VII and Porto Rico failed to produce harvestable roots. Only in La. IX were root lesions found, and following both methods of inoculation. Both methods resulted in lesions in 10% of the roots of this clone. Root development in the greenhouse

was typically poor because 5 months are needed to produce good size roots.

The Porto Rico plants inoculated in 1959 with leafspot virus only, showed only leafspot symptoms.

In brief, these studies indicated that internal cork virus was readily transmitted by both aphids and manual (micro-mechanical) methods; that two Porto Rico seedlings (La. II, La. IX) were more susceptible to the root-lesion phase of internal cork than the others tested (Fig. 1); that a leafspot virus was present in all inoculum used; and that the three sources of internal cork virus in 1957, whether from sweetpotatoes or Scarlett O'Hara indicator plants, were essentially the same. Other studies indicate that feathery mottle virosis apparently involves internal cork, leafspot, and yellow dwarf viruses, (2, 3, 4).—ARS, U.S.D.A., Beltsville, Maryland.

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INHERITANCE OF RESISTANCE IN CUCUMBER TO CUCUMBER MOSAIC VIRUS

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SUMMARY

The best greenhouse temperature for assaying cucumber for resistance and susceptibility to cucumber mosaic virus (CMV) was found to be 26-28°C when cotyledons were inoculated at the first leaf stage. The distinction between resistant and susceptible plants was determined at 20 days after inoculation on the basis of epinasty of the first true leaf, mottle of the newly developed leaves, and stunting of the plant. In the field, stunting was the major criterion of distinction. When commercial resistant varieties Wisconsin SMR12, Wisconsin SMR15, and Wisconsin SMR18 were crossed with susceptible varieties National Pickling and Wisconsin SR6, resistance was dominant in the F_1 generation. Progenies from F_1 plants crossed with the susceptible parents segregated at the ratio of approximately 1 resistant to 1 susceptible plant, while F_2 progenies segregrated close to the ratio of 3 resistant to 1 susceptible. This was also true in the greenhouse assay when Chinese Long, an Oriental resistant variety, was used as the resistant parent but modifying factors tended to obscure susceptibility in the field.

INTRODUCTION.—The purpose of this investigation was to study the inheritance of resistance in cucumber (*Cucumis sativus* L.) to the strain of cucumber mosaic virus (CMV) prevalent on this crop in Wisconsin. Porter (3) studied a cross between the resistant variety, Chinese Long, and the susceptible variety, White Spine, but was unable to fit his data into a factorial hypothesis. Shifriss, Myers, and Chupp (5) later studied material from a cross between Chinese Long and Early Russian and concluded that resistance as expressed by cotyledon symptoms was controlled by 3 complementary genes. With regard to leaf symptoms, they postulated 3 basic dominant resistant genes plus several modifying genes which were considered to be responsible for the fluctuation in degree of resistance noted. Resistance to the virus in Virginia Savoy spinach (*Spinacia oleracea* Mill.) was found by Pound and Cheo (4) to be dominant and monogenic. The same was reported by Sinclair and Walker (7) to be true for the resistant local-lesion reaction of cowpea (*Vigna sinensis* Savi.).

METHODS AND MATERIALS.—The virus strain used for all experiments was originally isolated from

spinach and designated as strain "Y" (2). It is similar in all known respects to the virus commonly collected from naturally infected cucumbers in Wisconsin. Stock cultures were maintained on plants of *Nicotiana glutinosa* L. in insect-proof cages. Inoculum was produced on plants of Small Sugar pumpkin (*Cucurbita pepo* L.); cotyledons were inoculated when the first true leaf had reached about half normal size in a greenhouse maintained at 26-28°C. Assay of virus titre in such plants on Black cowpea showed that it varied in cyclic fashion as in tobacco (1,9), reaching the first peak in systemically infected leaves at 12 days and the second peak at 20 days after inoculation. To prepare inoculum, the 3 youngest systemically infected leaves were macerated, the extract filtered through a double layer of cheesecloth, diluted 1:10 with distilled water, and inoculations were made immediately. In greenhouse experiments, unless indicated otherwise, the inoculations were made on cotyledons of cucumber when the first true leaf was about half normal size. Cotyledons were dusted with 600-mesh Carborundum and the entire upper surfaces were wiped with cheesecloth soaked in the inoculum. In field experiments, inoculations were made similarly on cotyledons at the first to third leaf stage.

In the greenhouse, seeds were germinated in vermiculite and seedlings were transplanted individually to 3- or 4-in. pots of compost soil. In the field, seeds were planted in hills 5 ft apart in 6-ft rows and seedlings were thinned to 2 or 3 per hill.

The susceptible parents used were National Pickling (NP) and Wisconsin SR6 (SR6). Resistant varieties used were Wisconsin SMR12 (SMR12), Wisconsin SMR15 (SMR15), Wisconsin SMR18 (SMR18), and Chinese Long (CL). The resistant character of SMR15 was derived from Chinese Long and Tokyo Long Green and that of SMR12 and SMR18 from Chinese Long (10, 11).

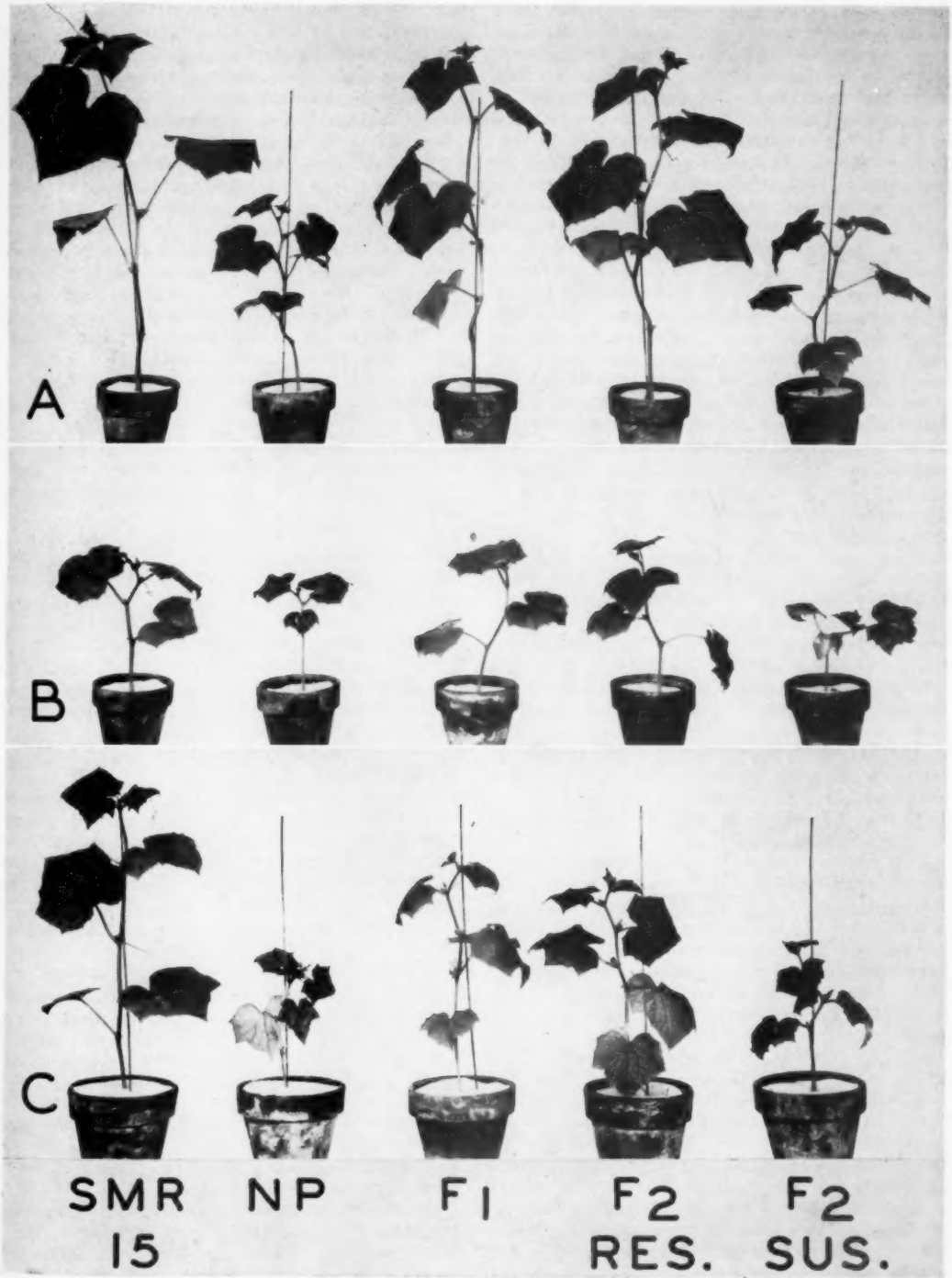
EXPERIMENTAL RESULTS.—*Development of the greenhouse assay.*—Sinclair and Walker (8) compared several resistant and susceptible varieties of cucumber under various combinations of temperature and day length in the greenhouse. They noted that the most consistent difference between the 2 types prevailed when plants were inoculated on cotyledons in the 3-leaf stage during spring and summer months and kept at 24-28°C. The least consistent differences were found when they were similarly inoculated in midwinter during short days of low light intensity. Both in the greenhouse and in the field, the separation of plants into discontinuous resistant and susceptible classes was complicated by the fact that all resistant plants became infected and showed symptoms when inoculated. Under field conditions, however, the symptoms in resistant varieties were mild and tended to disappear almost completely as the season progressed while in susceptible plants leaves remained smaller, mottle symptoms were more pronounced, internodes were shorter, and fruits when produced tended to be misshapen or severely mottled.

In the present investigation a study was first made of NP (susceptible), SMR15 (resistant), and F_1 and F_2 progenies between these 2 parental lines. Inoculations were made on cotyledons 6 days after transplanting when the 1st true leaf was about half normal size. This was at an earlier stage than that used by Sinclair and Walker (8). The parental lines and progenies were divided into lots of equal size; 1 lot of each was placed at 20°C and the other at 26-28°C. Certain of the plants of the parental lines were left uninoculated. At 26-28°C, the first striking difference between resistant and susceptible plants was very pronounced epinasty of the 1st leaf of susceptible plants usually on the 3rd day after inoculation. While mottle symptoms appeared on the youngest leaves of both resistant and susceptible plants at the same time, those of resistant plants expanded more rapidly and mottle was usually completely masked by the time leaves reached full size. Internodes of susceptible plants elongated much less than those of resistant plants. When compared with uninoculated plants the resistant plants showed some stunting, but the difference was much smaller than that between uninoculated and inoculated susceptible plants. At 20 days the most striking difference between resistant and susceptible plants was apparent. It was obvious that any attempt to place individuals of segregating progenies into resistant and susceptible classes would involve consideration of 3 symptom phases, i.e., epinasty, mottle, and stunting.

When F_1 plants were compared with parental lines, epinasty, mottle, and stunting were slightly greater than in those of the resistant parental line, indicating that resistance was incompletely dominant. However, F_1 plants recovered from symptoms rapidly and by the end of the 20-day period were nearly equal to parental resistant plants in height. In the F_2 progeny segregation was evident by the 5th day as extreme epinasty in about one-fourth of the population, such plants resembling closely those of the NP parental line. In the remainder of the F_2 population, plants resembled F_1 plants and parental resistant line plants. At this early stage, it was possible to pick out about one-fourth of the total

→

Fig. 1.—Representative plants of parental resistant Wisconsin SMR15 and susceptible National Pickling lines together with F_1 progeny and resistant and susceptible plants in the F_2 progeny, photographed 20 days after inoculation. **(A)** Plants inoculated on the cotyledons at the 1st leaf stage and kept at 26-28°C. Note that the F_1 and F_2 resistant plants are almost identical in height with the resistant parent plant. **(B)** Plants inoculated as in **(A)** but kept at 20°C. The difference between resistant and susceptible plants is much less than in **(A)**. **(C)** Plants inoculated on the cotyledons at the 3rd leaf stage and kept at 20°C. Incomplete dominance of resistance in F_1 and F_2 resistant plants is indicated as suppressed growth when compared with SMR plant.


A
B
C
SMR 15
NP
F1
F2 RES.
F2 SUS.

F_2 population on the basis of similarity to the parental resistant line, leaving about one-half of the population in an intermediate class which resembled the F_1 plants. By the 10th day the resistant and intermediate classes were practically indistinguishable. Thus, at the end of the 20-day period, the F_2 progeny was divided into 2 classes designated as resistant and susceptible, which contained approximately 75% and 25% of the population, respectively. Fig. 1-A shows representative plants incubated for 20 days after inoculation at 26°-28°C from the 2 parental lines, the F_1 progeny, and from the resistant and susceptible classes in the F_2 progeny.

In corresponding lots of inoculated plants incubated at 20°C, plants grew more slowly and symptoms appeared later. The distinction between resistant and susceptible parental lines was much less pronounced and it was not possible to separate individuals in the F_2 progeny into clear-cut resistant and susceptible classes. Representative plants from the various lots are shown in Fig. 1-B.

In another experiment, plants were inoculated in the 3rd true leaf stage and then incubated at 20°C. When inoculation was thus delayed, the expression of symptoms and the distinction between resistant and susceptible plants were closer to those described for plants inoculated in the early 1st leaf stage and incubated at 26-28°C. Representative plants from parental lines and F_1 and F_2 progenies are shown in Fig. 1-C. When these plants are compared with those in Fig. 1-A, it is evident that delayed inoculation at 20°C resulted in approximately the same degree of differentiation of resistant and susceptible plants as early inoculation at 26-28°C. It is to be noted, however, that the early but transient appearance of slightly incomplete dominance of resistance in F_1 plants at 26°-28°C was still evident 20 days after inoculation in F_1 plants at 20°C.

Since the early inoculation at 26-28°C required 26 days from transplanting for satisfactory assay as compared with 43 days for the delayed inoculation at 20°C, the former procedure was adopted as the standard for further testing of parental lines and hybrid progenies in the greenhouse. As inoculated plants were allowed to grow beyond the 20-day period in the greenhouse, the distinction between resistant and susceptible classes became less defined, probably due to the effect of growth factors which modified the expression of symptoms.

Development of the field assay.—Sinclair and Walker (8) pointed out that when resistant and susceptible varieties were planted out of doors in late May at Madison, Wis., and plants were inoculated in the 1st-3rd leaf stage, mottle was often delayed in appearance for 4-6 weeks while suppression of elongation of internodes in susceptible varieties was not conspicuous until after that time. In 1 of 3 seasons the difference in growth between the resistant and susceptible varieties studied was so slight that the difference in yield was not significant. The parental lines and the F_1 and F_2 progenies mentioned above were planted in the field and inoculated. Epinasty was not conspicuous. Leaf mottle appeared in resistant and susceptible parental lines at about the same time; as in the greenhouse, there was a greater tendency for mottled young leaves of resistant plants to mask symptoms as they expanded. The suppression of internodes in susceptible plants developed more slowly than in the greenhouse, while the early fruits on such vines often showed little or no mottle. The distinction between resistant and susceptible plants was thus more difficult to discern than in the greenhouse. The major criterion of distinction by mid-season was the relative length of internodes. In the segregating F_2 progeny, it was possible to distinguish about 25% of the plants which resembled the susceptible parent in this respect. There was a wider range of variation within this class in the field than in the greenhouse. This difference was undoubtedly due in part to the fact that the effects of modifying factors on expression of resistance were more completely suppressed in the greenhouse than in the field.

Assays of F_1 hybrid progenies.—F_1 hybrid progenies derived from SMR12 × NP; SMR15 × NP; SMR15 × SR6; SMR18 × NP; SMR18 × SR6 were assayed in the greenhouse and in the field. In the greenhouse tests, F_1 plants showed a temporary intermediate stage of disease when compared with the 2 parental lines, but at the end of the 20-day test period they were indistinguishable from plants of the resistant parental line. This early evidence of incomplete dominance was usually not observed in the field. The results from the assays are presented in Table 1.

Assays of F_2 progenies.—The F_1 plants of the 5 F_1 progenies listed in Table 1 were grown to maturity and F_2 progenies were tested in the field and in the greenhouse. The results are presented in Table 2. Under both assay procedures, progenies segregated consistently at the ratio of 3 resistant to 1 susceptible. These data together with the dominance of resistance in the F_1 progenies, pointed clearly to a monofactorial explanation of inheritance.

Assays of backcrosses of F_1 plants with susceptible parents.—Plants from each of the F_1 hybrid progenies listed in Table 1 were crossed with the respective susceptible parent to produce corresponding backcross progenies. The results from field and greenhouse assays are presented in Table 3. In conformity with the monofactorial hypothesis suggested by the data from the F_2 progenies, segregation of the backcross progenies was in each case a close fit to the expected 1:1 ratio.

Assay of F_3 progenies.—Sixty-eight F_2 plants from the cross SMR12 (resistant) × NP (susceptible) were grown and selfed. F_3 progenies were submitted to greenhouse and field assays. In the greenhouse, 18 were classified as homozygous resistant, 33 as segregating, and 17 as homozygous susceptible. This

was close to the expected 1:2:1 ratio. In the field it was noted, however, that while plants were fairly uniform within what appeared to be homozygous resistant and homozygous susceptible progenies there were distinct differences between susceptible progenies in size of plant. Also, it was more difficult to classify plants of segregating progenies into susceptible and resistant classes. This may have been due to the effect of modifying genes which accumulated in different proportions in the F_3 progenies. Of 3 progenies classified as segregating in the greenhouse, 1 was classified as homozygous resistant and 2 were classified as homozygous susceptible in the field.

Inheritance of resistance of Chinese Long cucumber.—As previously stated, the resistance of the parents used in the tests already reported was derived from Chinese Long or Tokyo Long Green varieties. Sill and Walker (6) reported that under optimum conditions for expression of mottle in young leaves in the greenhouse, these 2 varieties were distinct from American commerical resistant varieties in that they showed very little or no mottle. That such plants were systemically infected was demonstrated by the fact that the virus was shown to be abundant in the corolla tissue by the cowpea local lesion test. In this investigation, Chinese Long plants remained free from symptoms when inoculated in the field and in the greenhouse. F_1, $F_1 \times$ NP, F_2, and F_3 progenies from the cross Chinese Long $\times$ National Pickling were assayed in the field and greenhouse. In the greenhouse F_1 plants were shorter than those of Chinese Long and showed a diffuse mottle. At the end of 20 days, the plants had recovered from mottle and were very close to the resistant parental line in height. In the field the F_1 plants showed very slight mottle which was promptly masked and no suppression of growth was evident. In fact, the plants were larger than those of either parent. In the backcross progeny ($F_1 \times$ NP) tested in the greenhouse, 16 showed early epinasty and mottle and 16 were similar in reaction to F_1 plants. In the field 36 plants showed slight temporary mottle and no evidence of growth suppression while 31 showed mottle and suppressed growth, but the retardation was not as great as that in the NP parental line. When F_2 progenies were tested in the greenhouse, about one-fourth of the plants showed early epinasty and pronounced mottle on newly developed leaves and about the same number were free from mottle and epinasty. About one-half of

TABLE 1. Reaction of cucumber varieties resistant and susceptible to mosaic and F_1 hybrids from crosses between them when inoculated with cucumber mosaic virus in field and greenhouse

Variety or hybrid	No. of plants in resistant (R) or susceptible (S) class			
	Field assay		Greenhouse assay	
	R	S	R	S
Wisconsin SMR12 (resistant)	389	0	245	0
Wisconsin SMR15 (resistant)	120	0	124	0
Wisconsin SMR18 (resistant)	119	0	97	0
National Pickling (susceptible)	0	882	0	386
Wisconsin SR6 (susceptible)	0	141	0	51
SMR12 $\times$ NP	119	0	23	0
SMR15 $\times$ NP	45	0	80	0
SMR15 $\times$ SR6	52	0	20	0
SMR18 $\times$ NP	49	0	92	0
SMR18 $\times$ SR6	41	0		

TABLE 2. Segregation of F_2 populations from 5 crosses between mosaic resistant (R) and susceptible (S) cucumber varieties when assayed in field and greenhouse

Cross and type of assay	No. of plants in class indicated		χ^2 3:1	P range
	R	S		
SMR12[a] $\times$ NP[b]				
Greenhouse	64	25	.38	.70-.50
Field	102	38	.35	.70-.50
SMR15[c] $\times$ NP[b]				
Greenhouse	129	37	.64	.50-.30
Field	35	11	.03	.90-.80
SMR18[d] $\times$ NP[b]				
Greenhouse	180	68	.77	.50-.30
Field	44	15	.005	.95-.90
SMR15[c] $\times$ SR6[e]				
Greenhouse	92	30	.01	.95-.90
Field	50	15	.12	.80-.70
SMR18[d] $\times$ SR6[e]				
Field	40	14	.02	.90-.80

[a] Wisconsin SMR12 (resistant).
[b] National Pickling (susceptible).
[c] Wisconsin SMR15 (resistant).
[d] Wisconsin SMR18 (resistant).
[e] Wisconsin SR6 (susceptible).

TABLE 3. Segregation into mosaic resistant (R) and susceptible (S) classes of backcross progenies from F_1 plants crossed with the respective susceptible parent when assayed in field and greenhouse

Cross and type of assay	No. of plants in class indicated		χ^2 1:1	P range
	R	S		
(SMR12[a] $\times$ NP[b]) $\times$ NP[b]				
Greenhouse	12	8	.80	.50-.30
Field	61	53	.56	.50-.30
(SMR15[c] $\times$ NP[b]) $\times$ NP[b]				
Greenhouse	42	42	.00	.99-.98
Field	26	25	.02	.90-.80
(SMR18[d] $\times$ NP[b]) $\times$ NP[b]				
Greenhouse	74	73	.01	.95-.90
Field	21	18	.23	.70-.50
(SMR15[c] $\times$ SR6[e]) $\times$ SR6[e]				
Greenhouse	30	33	.14	.80-.70
Field	26	23	.18	.70-.50
(SMR18[d] $\times$ SR6[e]) $\times$ SR6[e]				
Field	23	24	.02	.90-.80

[a] Wisconsin SMR12 (resistant).
[b] National Pickling (susceptible).
[c] Wisconsin SMR15 (resistant).
[d] Wisconsin SMR18 (resistant).
[e] Wisconsin SR6 (susceptible).

the plants showed diffuse mottle which had disappeared at the end of the 20-day period. At this time 20 plants were classed as susceptible and 66 as resistant. In the field 36 plants were classed as susceptible and 120 plants as resistant. It appeared from these results that the same major dominant gene governed resistance as that which occurred in SMR12, SMR15, and SMR18.

Assay of progenies from SMR15 × SMR18.—The resistance in SMR12 and SMR18 was derived from Chinese Long; in the case of SMR15, Tokyo Long Green also was used as a resistant parent. This left open the possibility that a different gene for resistance was present in SMR15 than in SMR18 and SMR12. A cross was made between SMR15 and SMR18; and F_1, F_2, and F_3 progenies were assayed in the field. All plants were uniformly resistant, indicating that the same gene pair functioned in SMR12, SMR15, and SMR18.

Discussion.—The data presented indicate strongly that a single dominant gene is responsible for the resistance to CMV in the 3 commercial varieties studied and the resistant Chinese Long from which resistance has been derived in most resistant varieties developed in the United States of America. Since the type of resistance concerned is not of the exclusive or necrotic type, but is characterized by multiplication of the virus in both resistant and susceptible plants, it is to be expected that growth factors as influenced by environment would affect the expression of resistance and susceptibility. The widest difference between resistant and susceptible plants was found when seedlings were inoculated on the cotyledons when the first true leaf was about half normal size and incubated in the greenhouse at 26-28°C. At 20 days after inoculation the widest difference was evident. At longer intervals after inoculation and in plants where inoculation was delayed the distinction between the 2 classes was obscured. By using the early inoculation at 26-28°C as a standard, it was possible to determine that a dominant gene was responsible for the major resistant factor. This was confirmed in the field although the assay was not as reliable as in the greenhouse. It was obvious that modifying factors influenced the expression of symptoms and these were more pronounced in effect when Chinese Long was used as a parent than when the commerical varieties derived from it were used. It is quite possible that in the development of the American varieties the modifying factors in Chinese Long were dispersed or lost. The interference of such factors in greenhouse assays of older plants may account for the discrepancy between the present results and those of Shifriss, Myers, and Chupp (5).

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BOOK LISTINGS

Check List of Plant and Soil Nematodes. A nomenclatural compilation. 1960. Armen C. Tarjan. Univ. Florida Press, Gainesville. 200 p.

This book is an index to the taxonomy of plant-parasitic and soil-nematode species. In the author's words, "It attempts to present the proper spelling of each name, the correct authority for that name, most of the synonymies and homonymies that the taxa have undergone, and the current nomenclatorial status of each species." Names of species are listed alphabetically, with generic names and authorities, and synonymy indicated for each name. For each binomial, the reference is cited in which the name was first proposed. The book is an easily used and effective guide to the nomenclatural information on nematodes of interest to plant pathologists.

Traité de Botanique Systematique. Tome I. M. Chadefaud and L. Emberger. 1960. Masson & Cie., Paris. 1016 p. 713 fig.

Tome I of this 2-volume presentation of modern systematic botany covers cryptogamic plants. The first 2 chapters cover bacteria and blue-green algae; the next 4 cover red, brown, and green algae. Five chapters (500 pages) are devoted to the fungi. Mosses and bryophytes are handled in the fourth part of the book. A large number of rather diagrammatic illustrations emphasize the important features of the various systematic groups.

THE EFFECT OF AGE UPON SUSCEPTIBILITY OF EASTERN WHITE PINE TO INFECTION BY CRONARTIUM RIBICOLA

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SUMMARY

In tests of selections and their progenies for resistance to blister rust, the difficulty of obtaining infection in grafts of approximately 60-year-old trees was marked in comparison with severe infection of young seedlings. Infection responses to artificial inoculation of young seedlings, of grafts of young seedlings, and grafts of both resistant and susceptible selections of older trees were compared. Inoculations with basidiospores and by means of bark-patch grafts gave similar results. In all comparisons the percentage of infection of young seedlings was consistently much higher than that of grafts of considerably older trees. Grafting decreased susceptibilty to a limited extent, probably as a result of somewhat lower vigor of the grafts, as shown by 99% infection of 4-year-old seedlings compared with 81% infection of their grafts. A definite decrease in susceptibility with an increase in age was shown by the decreasing percentage of infection in grafts of 4-, 10-, 20-, 40-, and approximately 80-year-old trees as follows: respectively, 81, 76, 50, 42, and 27%. This indicated that caution should be exercised in comparing infection of grafts of parent selections and seedling progenies, but suggested that resistance which appeared in young seedlings would increase rather than break down with age.

INTRODUCTION.—An important problem in any program of breeding trees for disease resistance is effect of age on resistance. In testing grafts of selections and seedling progenies of eastern white pine (*Pinus strobus* L.) for resistance to white pine blister rust incited by *Cronartium ribicola* Fischer, differences in the susceptibility of grafts and seedlings to blister rust infection were evident. One major difference was the difficulty encountered in infecting grafts compared with the relative ease of obtaining a high percentage of infection of young seedlings by artificial inoculation. On seedlings, many more needle spots appeared than on grafts, more cankers per tree developed, and symptoms appeared much sooner. Heimburger (5) also reported that seedlings were much easier to infect than grafts.

Little direct information on the effect of age of the tree upon susceptibility to infection has appeared. Considerable interest was shown in possible differences in susceptibility of needles of various ages, and in the influence of such differences on cankering of both eastern and western white pines (*P. monticola* Dougl.). Much of the earlier literature on this question was summarized by Pierson and Buchanan (11). Studies on eastern white pine (though not entirely consistent) showed that needles of the current season's growth and those on 1-year-old internodes were about equally susceptible. In contrast, Hirt (6) found that the majority of cankers on young trees developed on needle-bearing wood that was 1 year old when exposed to natural infection. With western white pine, needles of the current season appeared to be relatively low in susceptibility, and needles of the 2nd and 3rd seasons relatively high and approximately equal in susceptibility (8, 11).

Most reports concerning differences in susceptibility of trees of different ages pertained to young seedlings differing in age by only a few years (2, 14, 16). Inoculation in the field of entire trees 5-15 years old or of the lower branches of trees 15 to approximately 30 years old resulted in infection of 29% of the trees in the 1st group, and 36% in the 2nd (14). No small seedlings were included as controls, although previous inoculation trials with young potted seedlings usually resulted in infection of well over 50% of the trees (16).

Other reports on potential blister rust damage to various natural stands (1, 7, 12, 13) yielded conflicting evidence from observations of natural infection.

This paper presents information on age and susceptibility to blister rust infection gathered during the course of investigations on the development of blister-rust-resistant eastern white pine. The data were obtained from several resistance-screening tests and from one experiment designed specifically to test the effect of age and of grafting upon susceptibility to blister rust infection.

METHODS.—*Observations from resistance-screening tests of grafts and seedling progenies.*—1) *Spore inoculations.*—During the past 20 years, eastern white pines from 18-60 years old were selected for resistance (some for susceptibility) and propagated by grafting.

Seed, both from open and controlled pollinations, was collected from many of the selections and sown in the Griffith State Forestry Nursery. Grafts and 2-year-old seedlings were transplanted to test beds in the Blister Rust Nursery near Wisconsin Rapids where they were artificially inoculated with basidiospores of the blister rust fungus. In the fall, usually September or October, ribes leaves bearing good crops of telia were attached spore side down with paper clips or toothpicks to the current season's foliage, the beds were enclosed in muslin-covered frames, and the inoculation chambers then were kept wet by intermittent sprinkling for 60-72 hours. Controls in the beds were nursery-run seedlings the same age as seedling test stock. Many test lots were inoculated 2 and some even 3 times in successive years. The efficacy of the inoculation technique was shown by the resulting infection of 94-100% of the trees among different groups of several thousand seedlings inoculated. These inoculations were designed to test resistance of selections (as grafts) and of various seedling progenies. Infection records were taken annually. For the purpose of the present paper, an infected tree was considered as one that showed bark discoloration indicating establishment of the fungus in stem tissue.

2) *Bark-patch grafts.*—Since 1952 various trials have been made with bark-patch grafts in experiments to be discussed in another paper. A section of the bark tissue about 1¼-in. long was removed from the main stem or a branch, and a piece the same size and shape from the discolored bark of a blister rust canker was put in its place. The width of the patch was generally determined by the size of the stem from which the bark section was removed. Most patch grafts were made on 2- or 3-year-old internodes, but grafts were tried and were successful on stems ranging in age from current season's growth to the 3-in. trunk at breast height of a 15-year-old tree. The patch was held tightly in place by wrapping with a grafting rubber. On grafts made outside, an additional covering, such as masking tape, cloth grafting tape, or plastic electrical tape, was used to protect the rubber bands from checking and unwrapping. On grafts made in the greenhouse on potted stock, no such additional protection was necessary. The wrappings were left in place for at least 6-8 weeks before examinations were made. Successful transfer of the fungus from the infected bark patch to the stock was indicated by typical yellow bark discoloration, usually within 6-12 weeks. Progress of the infection was recorded over a period of 1 to several years. Patch grafts were made on potted seedlings 4 years old, on transplanted seedling stock 4-15 years old, and on grafts 1-15 years old made with scions from trees 25-60 years old.

Inoculation of grafts of trees of different age classes.—Trees for inoculation included control seedlings which were nursery run 2-2 white pine transplants, grafts with scions from 2-2 transplants, and grafts with scions from 10-, 20-, 40-, and approximately 80-year-old white pines in natural stands. Scions were taken from 5 different selections in each of the latter 4 age classes, and grafts were labelled by selection number and age class. Grafts were made in April, 1956, in the greenhouse; in June all plants were planted in the Blister Rust Nursery. Nine replicated plots (4 × 12 ft) were planted. The original plan was to have each age class (4-80 years) replicated 10 times, and each selection replicated twice; because of grafting and other losses, however, each age class was replicated 9 times, but not all individual selections were represented in every replication. Grafting losses were unusually high because grafting was done later and under less favorable conditions than normally. The plan called for 160 control 2-2 seedlings and 30 grafts of each age class; subsequent to inoculation, infection records could be taken on 141 seedlings and only 11-21 grafts/age class. In each replication there were 16 control seedlings and 1-3 grafts of each age class. In October, 1956, and again in September, 1957, every tree was inoculated individually with 1 to several ribes leaves as described above. The data presented summarize all observations up to and including October, 1959.

RESULTS.—*Infection after spore inoculations of grafts and seedlings.*—Between 1940 and 1958 a number of resistance-screening trials provided data, summarized in Table 1, pertinent to the question of age and susceptibility. In all such trials, the percentage of young seedlings infected has been consistently much higher than of grafts made from considerably older trees.

In the 1st group of original Wisconsin selections, the trees have been rated and roughly divided into 2 groups as resistant or susceptible according to their response both to natural and artificial inoculation over a period of years. The selections probably represent trees varying from susceptible to highly resistant. The percentage of infected trees was the average of that in replications given artificial inoculation and in replications exposed only to natural infection. The response differed between the resistant and susceptible groups of grafts, both showing less infection than seedlings, but even selections rated as susceptible had only about half as many trees infected as the seedlings. Most of the selections were within the range of 28-50 years of age.

In the 2nd and all subsequent groups in Table 1, all trees were subjected to 1 or more artificial inoculations. The grafts in the 23- and 40-year-old groups were from trees originally chosen for possible rust resistance (Group 1—Wisconsin selections, Table 1); although, from infection responses after inoculation in the nursery, they were judged to be at least fairly susceptible. The percentage of infected grafts of trees with an average age of 40 years was 18% less than of grafts of trees averaging 23 years. The infected controls were selected in the same area as the 23-year-average group, but bore active blister rust cankers. Thus, these trees were slightly younger and possibly also inherently more susceptible to infection than the other 2 groups. The percentage of infection on these

control grafts was the highest ever obtained on grafts of field selections. Inoculations of these trees and of the other 2 groups of grafts were in different years, but even if this were in part responsible for different amounts of infection in the groups of grafts (besides differences in susceptibility and in age), the percentage of infected grafts of the older trees was still considerably less than the amount of infection in the 2 lots of control seedlings.

The selections from Duluth, Minn., came from a heavily infected plantation in which records on the parent selections were continued during subsequent testing of grafts. From these records, plus the response of grafts to artificial inoculation, the selections were rated as resistant or susceptible. Although differences between the two groups of grafts again were evident, the marked difference in the amount of infection obtained with the grafts compared with that of seedlings was consistent with similar tests.

The selections from Beaver Bay, Minn., provided one of the most significant comparisons. When the resistant selections were chosen, 6 equivalent but severely infected trees were selected from the same stand as controls. Resistant and susceptible selections were randomized and replicated in 9 test beds, and control seedlings were planted in each bed. The trees were planted in May, 1951; inoculated in September, 1951; and again in September, 1953. The percentage of infection ranged from 0-19% for the resistant selections represented by 9-18 grafts each. Infection of 4 selections (9-16 grafts each), which later examinations showed were susceptible, ranged from 19-50% for the individual selections, with 3 of the selections having 40, 45, and 50% infection. Infection of the 6 known susceptible control selections (5-14 grafts each) ranged from 11-83%. Again, there was a difference between resistant and susceptible selections, but both had much less infection than the control seedlings.

The known susceptible selections, made in Wood County, Wis., were included as controls in a test of some exotics. The distinct contrast between old and young susceptible trees again was expressed after 2 inoculations.

Infection with bark-patch grafts.—During 1952 to 1960, a series of bark-patch grafts with infected bark tissue was made for various purposes. The results paralleled those obtained from spore inoculations of seedlings and of grafts of older trees. Results of the various series have been combined to show the total percentage of infection on the 2 types of stock. From 449 patch grafts on grafts of 25-60-year-old trees, there were 14% infections; from 380 patch grafts on young seedlings (mostly 4-year-old potted stock), there were 70% infections.

Infection of grafts of trees of different age classes.—Since all of the experiments above involved comparisons of grafts of older trees with young seedlings, the role of grafting confounded the role of age. An obvious solution was a comparison of artificial inoculations in the field on both young and old trees. A number of preliminary trials of field inoculation with various techniques, however, had not been successful, since only a low percentage of infection was obtained even on seedlings. Therefore, the trees, in the form of grafts, were brought to the disease nursery, where

TABLE 1. Infection by *Cronartium ribicola* after artificial inoculation of control seedlings and grafts of resistant and susceptible white pine selections

Stock tested	Grafts		Seedlings	
	No. inoculated	% infected	No. inoculated	% infected
1. Wisconsin selections [a]				
Grafts—"resistant" selections	284	21		
Grafts—"susceptible" selections	772	50		
Open-pollinated progenies			5084	94
Control nursery seedlings			2003	91
2. Susceptible selections, age-class groups				
Grafts of selections, avg. age 40 yrs.	243	50		
Grafts of selections, avg. age 23 yrs.	389	68		
Control seedlings			1009	99
Grafts, infected controls, avg. age 18 yrs.	49	80		
Control seedlings			120	100
3. Duluth selections—age 35 yrs.				
Grafts, 9 resistant selections	46	30		
Grafts, 14 susceptible selections	60	50		
Control seedlings			29	96
4. Beaver Bay selections—age 60 yrs.				
Grafts, 18 resistant selections	253	8		
Grafts, 4 discarded susceptible selections	49	37		
Grafts, 6 susceptible controls	53	34		
Control seedlings			122	91
5. Wood County selections—age 60-80 yrs.				
Grafts, 3 susceptible controls	15	20		
Control seedlings			18	100

[a] In this 1st group some trees were subjected to only natural infection over a 13-year period, and others were artificially inoculated. All other groups were artificially inoculated.

it was possible to employ a relatively efficient inoculation technique, and effects of both age and grafting were considered.

TABLE 2. Infection after artificial inoculation of 4-year-old white pine seedlings and of grafts of white pines in various age classes

	Age (years)	Total no.	No. infected	% infected
Control seedlings:	4	141	139	99
Grafts:	4	21	17	81
	10	17	13	76
	20	16	8	50
	40	12	5	42
	80	11	3	27

Records of infection indicated that there were no significant differences in susceptibility among the individual selections within each of the various age classes, and so the data were combined as summarized in Table 2.

The effect of grafting upon susceptibility to blister rust infection was shown by the comparison of infection on 4-year-old seedlings and on grafts with scions from 4-year-old seedlings. The difference in infection ($\chi^2 = 11.31^{**}$) indicated that grafting in itself did lower somewhat the susceptibility of the test material. The difference between the proportion of infections of grafted and nongrafted stock of the same age, however, was not as great as the usual difference encountered with seedlings and grafts of much older trees.

The effect of age on susceptibility was shown by the comparison of infection on grafts of trees in 4-80-year-old age classes. Infection of the grafts decreased from a relatively high level in the 4-year-old class to a relatively low level in the 80-year-old class. The data were analyzed by the method illustrated by Steel and Torrie (15) for a test of a linear regression of p (proportion of infected trees). This analysis ($\chi^2 = 10.76^{**}$) showed that susceptibility to initial infection decreased as age of the tree increased. Infection of seedlings and grafts in this experiment was comparable to that obtained in other tests as seen in Table 1.

DISCUSSION.—Since most of the observations reported here were comparisons of seedlings and grafts, the effect of grafting had to be considered. The comparison of infection of grafts of 4-year-old trees with that of 4-year-old seedlings showed that grafting did reduce susceptibility to a limited extent. It is well known that vigorous hosts are more susceptible to infection by an obligate parasite, such as the blister rust fungus, than stunted, less vigorous plants. The most probable explanation for the lowered susceptibility of these grafts was the lower vigor of the graft compared with the seedlings. On grafted trees, growth of shoots and needles on the scion the 1st year after grafting was always stunted. Moreover, the top-root ratio of a graft was much different than that of a seedling of the same age; this difference was almost certainly reflected in the physiology of the tree. It is possible that this lowered vigor would extend over the 2- or 3-year period during which the artificial inoculations were made.

The influence of vigor on susceptibility could be expressed in the grafts of trees of different age classes. In comparisons of infection among the 5 age classes, it was assumed that the influence of grafting, if any, was equal in all the age groups, since all the trees were grafted similarly. If differences in susceptibility appeared, these could then be attributed to age of the scion tree. The age of the scion tree, however, could influence the effect of grafting through differentially affecting graft vigor. There are some suggestions that grafting is affected by age of the scion tree. Thus, from his experience with grafting western white pine, R. T. Bingham (personal correspondence) found that grafting success and vigor of scions decreased with an increase in scion tree age. In the present work with eastern white pine, clonal differences have been much more noticeable than any differences among age classes either in grafting success or in subsequent growth of grafts. Vigor (whatever is encompassed in the term) might well be affected by age of the scion tree, but rather than strength or force of normal physiological activities of the tree (as expressed by vigor) affecting susceptibility, the nature of physiologic changes due to age seems of more importance. Of much greater significance than grafting as such apparently is the effect of the physiological age of the scion.

The phenomenon of physiological aging is little understood, but there is no question that it exists. Its characterization is usually on the basis of flowering, rootability, or simple morphological features, but the fundamental changes in aging are centered within the plant tissues (9). An example of the influence of aging in white pine on some associated character is the sharp and rapid decline in rooting ability of this species with an increase in age (3, 4, 10). Also notable is the difference in growth habit of juvenile and mature growth phases of the tree. Grafts of older trees for the first several years typically have fewer needles per unit length of stem and stems of smaller diameter; thus, the grafts have a slender, spindly aspect in contrast with the more bushy and heavier-branched appearance of seedlings. It is evident that physiological differences exist between the so-called juvenile and mature growth phases of white pine. Such differences could just as well affect susceptibility to blister rust as rootability. The nature of the differences, however, has not been defined.

Neither needle age nor relative amount of needles on trees of different ages can be considered relevant to the question of influence of age on susceptibility from the standpoint of data presented here. Both factors are of some significance in evaluating infection in the field under natural conditions, where the amount of target area exposed to possible contact with spores in the air would be important, as well as any alteration of the microclimate effected by type or amount of needles present. In the experiments described above,

practically all inoculations were made on needles of the current season. The size of the target was not a factor in affecting infection since spores were applied directly to the needles in a localized portion of every tree, usually at the top of the current season's leader, or sometimes near the tip of the current season's lateral branches.

Since the infection criterion used here was the establishment of the fungus in stem tissues as indicated by bark discoloration, differences in infection in trees of different ages could have been due either to differences in susceptibility of the needles to initial infection, to varying abilities of host needles to support fungus growth down to the stem, to differences in susceptibility of the stem bark tissues to infection, or to a combination of these. It was well established by much of the early blister rust work that the incubation period (the time between infection and bark discoloration) is usually a whole year longer on older trees than on seedlings under about 4 or 5 years old. Thus, the cycle of disease development is known to be affected by age, and it appeared even more true of susceptibility to initial infection.

Apparently, changes brought about through the physiological aging process extended both to needle and bark tissue. After the artificial inoculations described above, a lower percentage of grafts than seedlings showed needle spots, indicating that needle susceptibility was affected by age. The results of bark-patch graftings, which bypassed the needles, were consistent with results of basidiospore inoculations of needles, and indicated that bark tissue susceptibility also decreased as host age increased. Thus, it seems likely that there is an over-all change in host tissue susceptibility and not a localized change in some specific tissue.

Increase of resistance to infection with age does not mean, of course, that the trees cannot be infected. For example, trees chosen as susceptible controls in the field along with apparently resistant selections had one or more girdling trunk cankers and several lateral branch cankers. This infection, however, was the result of at least 20 years' exposure to abundant blister rust inoculum in an area where conditions for infection are often favorable during the period of telia production. The disparity was marked between the relatively low percentage of infection after artificial inoculation of single clone members in such a tree and in a much younger tree. This response indicates that there must be a higher threshold for infection in older trees than in younger trees.

This poses several questions which appear to bear on this problem. What is the influence of amount of inoculum in causing infection of pines of different ages? What is the degree of variation in pathogenicity of individual basidiospores? How do initial infection and disease development resulting from a single basidiospore compare with those from a mass of basidiospores? Pine susceptibility as influenced by tree age would be closely related to all these questions. One reasonable hypothesis for test (in explanation of the different response of young and old trees to artificial inoculation) is this: in any given quantity of basidiospores there are spores with varying infection capabilities and the number of spores capable of causing infection of older trees is less than that for young trees. Thus, infection of a susceptible young tree might be caused by any of a relatively large number of spores, whereas infection of a tree of similar genotype, but many years older, might be caused by only a relatively few spores in that same group.

The increase in resistance to infection with increase in age has a number of implications in a program of development of disease resistance. For example, caution must be exercised in making comparisons between infection of grafts of old parent selections and of seedling progenies. Also, it can be expected that progenies of resistant parents, regardless of their inheritance of factors for resistance, will be more susceptible in their younger stages than the parents. This poses the question of when progeny testing should be done. The time element is a constant problem in any tree breeding program; short cuts in progeny testing procedures are most desirable, particularly in a program such as blister rust resistance where the disease cycle is so long. The danger of elimination of much potentially valuable material through severe screening tests has long been recognized, but the method is accepted as a conservative and safe procedure. Perhaps the same might be said for screening progenies in their most susceptible stages, but at least the possibility of loss of breeding stock must be recognized. On the brighter side is the consideration that if resistant trees are found in tests of young stock, their resistance may be expected to increase rather than break down with increasing tree age.

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RADISH MOSAIC VIRUSES

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SUMMARY

Parallel studies were made with the radish mosaic virus of Severin and Tompkins, several virus isolates from radish and other hosts in California, and known members of the turnip mosaic virus and cauliflower mosaic virus groups. Isolate RMV 1, the radish mosaic virus used by Severin and Tompkins, had the same host range, symptoms, thermal inactivation point, host immunity reactions, and virus particle morphology as cabbage virus B, a known strain of cauliflower mosaic virus. Of the other isolates collected in California, some were of the cauliflower mosaic virus group and some were of the turnip mosaic virus group. Results indicate that the radish mosaic virus of Severin and Tompkins was not the same as that originally described by Tompkins. This questions the identity of the radish mosaic virus purified by Takahashi and the validity of Sylvester's classification of the radish mosaic virus, a classification based largely on the papers of Takahashi and of Severin and Tompkins.

Introduction.—Most of the aphid-transmissible crucifer viruses have been placed in 2 unrelated groups, turnip mosaic virus (2) and cauliflower mosaic virus (15), by studies of host range, physical properties, and immunology (6, 7, 9, 10, 13). Strains of turnip mosaic virus have a wide host range, a thermal inactivation point of about 60°C, and virus particles that are long, flexuous rods (6, 12). Strains of cauliflower mosaic virus are limited to the Cruciferae, have a thermal inactivation point of about 75°C, and have recently been shown to consist of spherical particles (1, 8).

In 1939, Tompkins (16) described a mosaic virus of radish that had a thermal inactivation point between 63° and 68°C but was not aphid-transmitted. In 1950, however, Severin and Tompkins (11) reported that this virus was easily transmitted by aphids.

These discrepancies in aphid transmissibility and in descriptions suggested that more than one virus was concerned. Tompkins (16) described the first symptoms as "small, roughly circular to irregular chlorotic lesions," whereas Severin and Tompkins (11) described them as "a clearing of the veins and veinlets, first appearing on the outer or intermediate leaves and spreading to the youngest leaves, followed by yellow veinbanding." The reader may assume that the authors thought they were working with the same virus and that the inoculum from both studies originated from the same source. Some of the illustrations of Severin and Tompkins were obviously made from the negatives used by Tompkins, although reversed and enlarged.

Takahashi (14) reported purification of the radish mosaic virus and described the virus particles as short, rigid rods. He obtained the virus that he purified from Tompkins and undoubtedly assumed it was the original Tompkins virus. On the basis of short, rigid rods described by Takahashi, the aphid transmission reported by Severin and Tompkins, and other properties, Sylvester (13) erected the Radish Virus 1 group to accommodate the radish mosaic virus and the virus of the stunt disease of radish described from Japan (4, 5).

This paper reports a comparative study of several radish viruses in parallel experiments with known

viruses of the turnip mosaic and cauliflower mosaic virus groups in an attempt to elucidate the relationship of the radish mosaic virus to these groups. A preliminary report has been made (3).

MATERIALS AND METHODS.—The sources of the viruses used were: cabbage A virus (CAV), G. S. Pound; cabbage B virus (CBV), G. S. Pound; cabbage black ring virus (CBRV), G. S. Pound, originally from C. M. Tompkins; radish mosaic virus (RMV 1), W. N. Takahashi, originally from C. M. Tompkins; radish mosaic viruses (RMV 2, RMV 3), R. G. Grogan, from wild radish in central California; radish mosaic virus (RMV 4), A. O. Paulus, from commercial radish in southern California; radish mosaic virus (RMV 5), L. G. Weathers, from commercial radish in southern California; radish mosaic virus (RMV 6), A. O. Paulus, from commercial radish in central California; wild mustard virus (WMV), A. O. Paulus, from wild mustard in southern California.

Stock cultures of these viruses were maintained in a desiccated state at 4°C. Inocula for day-to-day experiments came from rape (*Brassica napus* L. var. Dwarf Essex), radish (*Raphanus sativus* L. var. White Icicle), or *Nicotiana glutinosa* L. plants infected from desiccated inoculum. Before inoculation by rubbing, Carborundum (600 mesh) was applied to the leaves with a DeVilbiss powder blower. After inoculation, the leaves were rinsed with water and the plants removed to the desired air temperature.

In host trials, parallel inoculations were made with all radish mosaic viruses, CBRV, CBV, and CAV, at constant greenhouse temperatures of 16° and 28°C. For each virus and each temperature, 5 or more plants were inoculated and an equal number of healthy plants retained as checks. RMV 6 was received too late for most of the experiments.

For host immunity studies, rape, radish, and *Nicotiana multivalvis* Lindl. were used as hosts for systemic development, and *N. tabacum* L. var. Havana 38 or *N. glutinosa* was used as an assay host. The radish mosaic viruses were inoculated to a susceptible host, which was incubated at 24°C until definite systemic symptoms were observed. Half of the infected plants and an equal number of healthy plants of the same age were then supra-inoculated with CBRV or CAV. After systemic development of CAV or CBRV in the healthy plants was evident, equal weights of the very youngest leaves of singly and doubly inoculated plants were triturated with water to give a 1:15 (w/w) dilution and assayed in half-leaf inoculations on tobacco plants on which CAV and CBRV gave diagnostic necrotic lesions. A randomized complete block design was used. In later experiments, assays were made to 2 *N. glutinosa* plants, which were then incubated at 16°C. At that temperature, CBRV produces severe systemic symptoms that can be easily differentiated from those of the radish isolates. This procedure was necessary for isolates that produced local lesions on tobacco, similar to those of CBRV or CAV.

Thermal inactivation determinations were made with undiluted, expressed sap in thin-walled glass pipettes submerged in a thermostatically controlled water bath. After 10 minutes in the water bath, the pipettes were chilled quickly and the contents mechanically inoculated to susceptible hosts. All viruses were tested 3-6 times on radish except WMV, which was tested on rape.

Insect transmission studies were made with the green peach aphid, *Myzus persicae* (Sulz.), and the cabbage aphid, *Brevicoryne brassicae* (L.). Rutabaga and cabbage plants grown from seed were used as culture hosts for the aphids. In early experiments, single aphids starved for 2 hours were permitted to feed for 10 minutes on an infected radish plant. Then they were transferred to a healthy radish plant for an infection feeding of 24 hours, and killed. In later experiments, groups of 25 or 50 aphids were collected with an aspirator and starved for 2 hours. After an acquisition feeding of 10 minutes, the aphids were disturbed with a strong light and collected with an aspirator as they stopped feeding. They were then placed as a group upon a very young radish plant, allowed an infection feeding of 24 hours, and killed.

RESULTS.—*Reactions on cruciferous hosts.*—On White Icicle radish, these viruses produced 2 types of symptoms:

One type, produced by RMV 1 and 3, and CBV, was a veinclearing (Fig. 1-A) that appeared on youngest leaves, in 6-10 days at 28°C, and in 12-16 days at 16°C. Yellow veinbanding and interveinal chlorosis developed as the leaves matured.

A systemic coarse veinbanding was frequent, accompanied by dark-green bands parallel to and between the veins (Fig. 1-B). The entire leaf was bleached to a bright yellow by interveinal chlorosis, except for this dark-green veinbanding. The interveinal chlorosis occasionally resulted in dark-green raised or blister-like areas along the veins (Fig. 1-C). Plants were malformed and distorted. At the lower temperature, veinclearing was persistent, changing finally from a fine to a coarse appearance, and only occasionally being replaced by a yellow veinbanding. Marginal chlorosis and the production of dark-green, blister-like areas were never observed at this temperature.

The other type of symptom, produced by isolates RMV 2, 4, 5, 6, CAV, and CBRV, was a systemic mottle. It first appeared in 6-10 days at 28°C, and in 10-14 days at 16°C. Initially, symptoms were either a mild mottle (Fig. 1-F) or a fine veinclearing that rapidly changed to mild, diffuse mottle. Succeeding leaves were stunted and distorted, and showed a severe blotch-type mottle, often with raised dark-green islands on a bleached yellow background (Fig. 1-D,E). Chlorotic spots were common on inoculated leaves, and occasional on systemically invaded leaves. At 16°C the symptoms were the same, but developed more slowly.

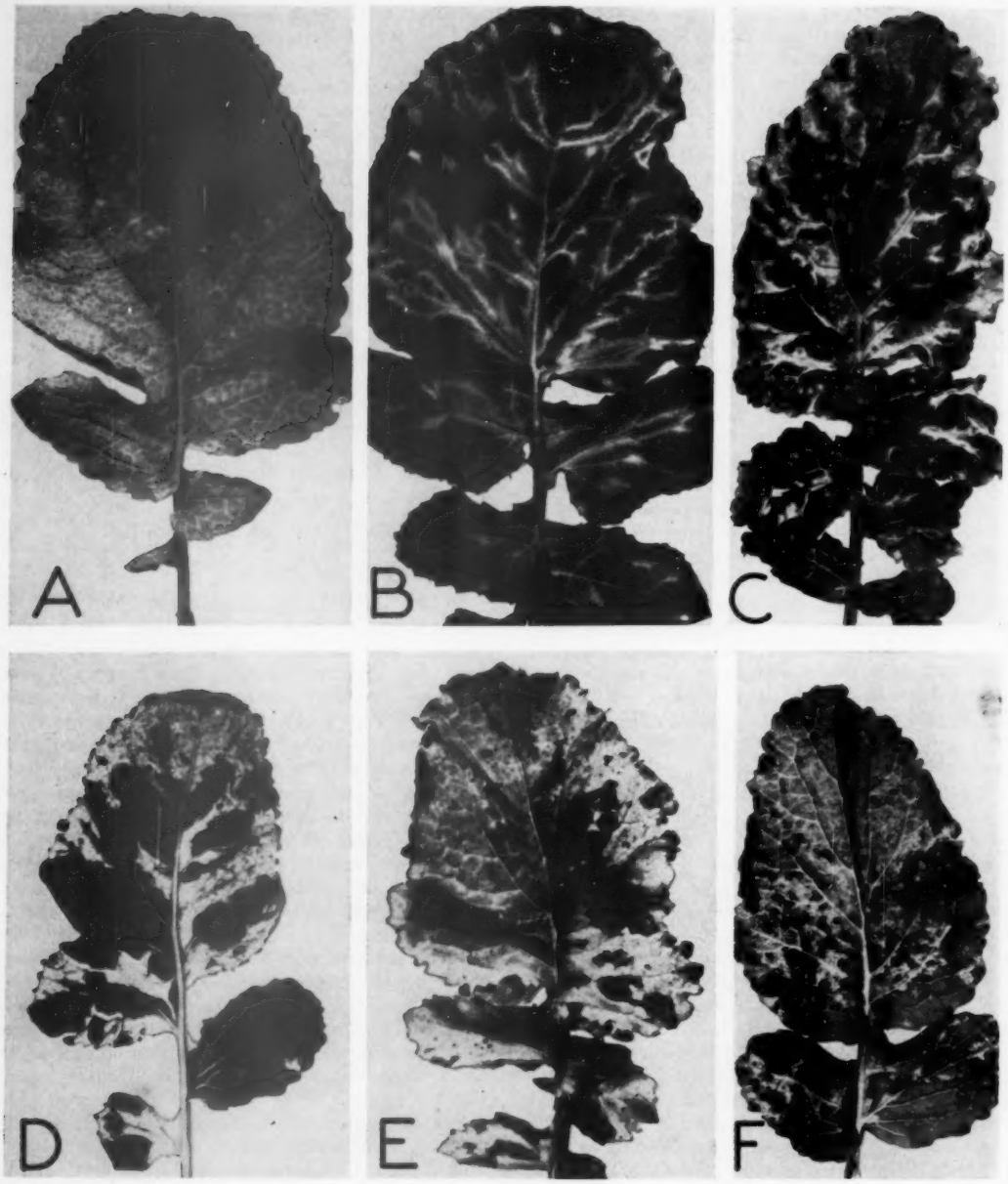

Fig. 1. Systems produced on White Icicle radish by 2 groups of virus isolates. **(A-C)** Veinclearing and veinbanding produced by RMV 1. **(D-F)** Blotchy mottle and severe chlorosis produced by RMV 4.

Two trials were made with a number of common radish varieties. Only isolates RMV 1, 3, and CBV caused symptoms on all varieties; the other isolates were erratic in causing symptoms. WMV did not produce symptoms on any variety.

The viruses could not be separated from one another or into groups on the basis of symptom differences on *Brassica rapa* L., turnip, var. Purple Top White Globe, and Shogoin; *B. juncea* (L.) Coss, leaf mustard, var. Tendergreen; and *B. campestris* L., Wild Yellow Mustard. Symptoms of veinclearing and mottle were found in all cases, and although plant death was

usually earlier from isolates RMV 2, 4, and 5, WMV, CAV, and CBRV than from RMV 1, 3, and CBV, the time difference was variable.

Reliable separation of 2 groups could be made by symptoms on other crucifers. On rape var. Dwarf Essex, isolates RMV 1, 3, and CBV caused a veinclearing that became coarse and persistent. In contrast, isolates RMV 2, 4, and 5, WMV, CAV, and CBRV produced local necrotic lesions (RMV 2, 4, and WMV only) and/or systemic mottling, stunting, and distortion. Differences were observed between members of this group in apparent incubation period and severity, but no deviations from the general symptom patterns were found. In general, the symptoms on rape were paralleled by those on *Brassica napobrassica* Miel., rutabaga, var. American Purple Top; *B. pekinensis* Rupr., Chinese cabbage, var. Michihli (Fig. 2); *Matthiola incana* var. *annua* Voss., stock var. Dwarf Large Flowering Ten Weeks; and *B. oleracea* var. *capitata* L., cabbage var. Jersey Queen. Exceptions were on Chinese cabbage—a veinal necrosis produced by RMV 2, and extremely severe mottling and stunting caused by WMV. Symptom differences on stock were less distinct at 28° than at 16°C, and some transient veinclearing occurred with isolates WMV, CAV, and CBRV. All isolates induced flower breaking on this host. On cabbage, only CBRV produced necrotic lesions.

Noncruciferous hosts.—Isolates RMV 1, 3, and CBV did not infect any noncruciferous host. Consequently, only results with RMV 2, 4, 5, and 6, WMV, CAV, and CVRV are reported.

On *Zinnia elegans* Jacq., zinnia var. Golden Giant, symptoms of isolates RMV 2, 4, 5, and 6, WMV, CAV, and CBRV consisted of initial faint veinclearing of the very young leaves, followed by a coarse mottle. Infected plants were usually stunted.

On *Chenopodium album* L., lambsquarters, initial local chlorotic lesions and later systemic small chlorotic spots were produced by all isolates except RMV 4 and 5.

All isolates produced an initial veinclearing and a systemic mottle on *Petunia hybrida* Vilm., petunia var. Dwarf Red.

On *Solanum integrifolium* Poir., Chinese scarlet eggplant, differences between isolates were large. The usual pattern of early veinclearing followed by a coarse mottle was observed, but certain isolates caused early death of the plant while others caused only mild symptoms. At 28°C, isolate RMV 5 caused plant death in 9-12 days, RMV 4 killed plants 3-4 days later, RMV 2 caused extreme dwarfing, CAV caused moderate dwarfing, and WMV caused only mild chlorotic or necrotic spotting. CBRV was not infectious to this host.

On *Nicotiana rustica* L., all isolates produced occasional chlorotic spots on inoculated and systemically infected leaves. On *N. multivalvis* Pursh., CBRV caused small, whitish rings, locally and systemically, with severe stunting and death. The other isolates caused varying degrees of mottle, distortion, and stunting. Differences were similar but not identical on *N. langsdorfii* L. On *N. tabacum* L., tobacco var. Havana 38, local necrotic lesions of varying types and intensities were formed. On *N. tabacum* var. 423 and an F_1 hybrid of *N. tabacum* × *N. glutinosa*, a similar situation occurred in the production of primary lesions. CAV and CBRV regularly produced distinct necrotic lesions, whereas RMV 4, 5, and WMV produced only chlorotic lesions (Fig. 3). At 28°C all isolates caused local symptoms of concentric white rings on *N. glutinosa* L. At 16°C on this same host, large differences existed in the incubation periods of various isolates and their ultimate effect on the host. Isolate CBRV killed the plants in 28 days, much earlier than any other isolate. Isolates RMV 4, WMV, and RMV 2, respectively, caused severe, moderate, and extremely mild mottle symptoms.

Thermal inactivation determinations.—Thermal inactivation points for all isolates are given in Table 1. The isolates were of 2 distinct groups: RMV 1, RMV 3, and CBV, in 1 group (surviving at 70-75°C); and all other isolates in the other (destroyed at 60-65°C).

Host immunity studies.—In early experiments rape was used as a systemic host, and isolates RMV 1, 2, 3, 4, and 5, WMV, and CBV were used for the 1st inoculation. CAV was the challenge virus, and assays were made on tobacco. In later experiments *N. multivalvis* and rape were used as systemic hosts, and assays were made on *N. glutinosa*. Composite results are given in Table 2. RMV 1, RMV 3, and CBV did not cross protect against CAV or CBRV, whereas all other isolates did cross protect against CAV and CBRV.

Insect transmission tests.—Early experiments with single aphids produced extremely erratic results. In later experiments with groups of 25-50 aphids on a single susceptible host, 100% transmission was achieved with all isolates, by both *M. persicae* and *B. brassicae*. Resulting symptoms from transmission tests were identical to those on the source host.

Purification of RMV 1.—Following the technique used by Pirone, *et al.* for purification of the cauliflower mosaic virus (strain CBV), the RMV 1 isolate was readily purified from Tendergreen mustard plants. The virus particles were spherical and about 50 mμ in di-

TABLE 1. Effect of 10-minute heat treatments of extracted sap on infectivity of crucifer viruses

Isolates	No. of trials	Avg. no. of plants infected, of 5 inoculated at (°C): 50	55	60	65	70	75
RMV 1	6	5	5	5	5	5	2
RMV 2	5	5	5	2	0	0	0
RMV 3	3	5	5	5	5	2	0
RMV 4	6	5	5	2	0	0	0
RMV 5	3	5	5	2	0	0	0
RMV 6	1	5	5	1	0	0	0
WMV	3	5	5	2	0	0	0
CAV	3	5	5	1	0	0	0
CBV	3	5	5	5	5	5	2
CBRV	3	5	5	1	0	0	0

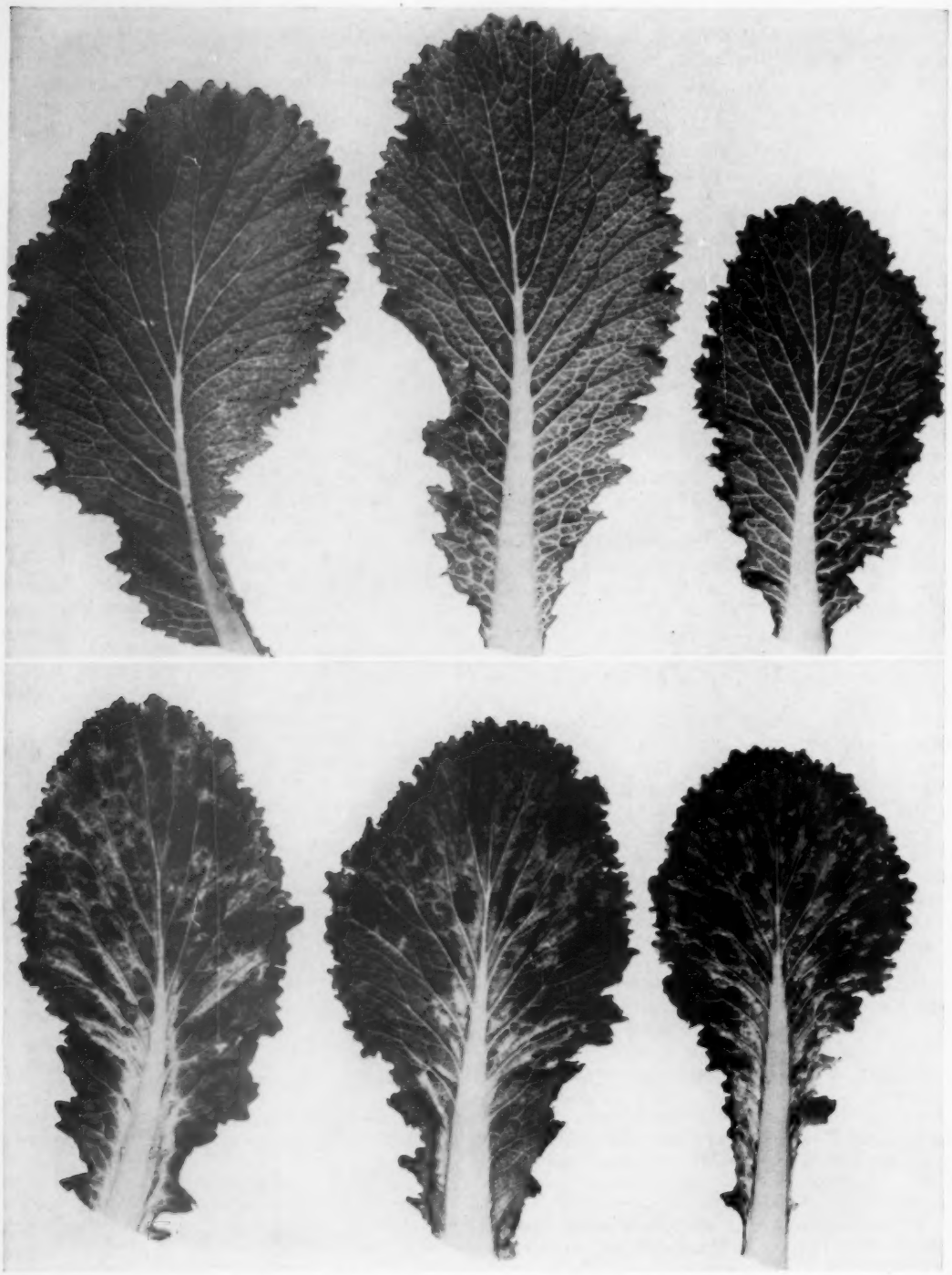

Fig. 2. Symptoms produced on Chinese cabbage by crucifer virus isolates. Top (left to right), RMV 3, CBV, and RMV 1. Bottom (left to right), CAV, RMV 5, RMV 4. Note the characteristic veinclearing above, and the blotchy mottle below.

Fig. 3. Primary lesions produced on inoculated leaf of *Nicotiana tabacum* by CBRV (right half) and WMV (left half) virus isolates. Isolates RMV 4 and 5 also produced chlorotic lesions similar to WMV.

ameter. The purified virus particles appeared to be identical to those of the CBV strain of the cauliflower mosaic virus. Takahashi's purification procedure was followed in many attempts, and in no instance were short, rigid rods obtained.

DISCUSSION.—Much present confusion in the differentiation and classification of crucifer viruses has resulted from inadequate definition. Many of the original virus cultures have been lost and can not now be used to determine strain relationships. This was the case with the radish mosaic virus. Tompkins' original description was made in 1939; when Severin and Tompkins showed aphid transmission in 1950, they presumably used the original virus, as did Takahashi in 1952, when he reported its purification. The suggestion by Sylvester of a special taxonomic group to accommodate the radish mosaic virus was based on the results of those workers.

When RMV 1, the virus received from Takahashi and presumed to be the original radish mosaic virus, was tested on the same host under approximately the same temperature conditions used by Tompkins, the symptom development paralleled that described by Severin and Tompkins, and disagreed with Tompkins' original description on the same points as did the description of Severin and Tompkins. CBV, a known strain of the cauliflower mosaic virus, produced symptoms on this host almost identical with those of RMV 1, whereas CBRV and other known strains of the turnip mosaic virus caused symptoms similar to Tompkins' original description. It must be concluded that RMV 1 and the original radish mosaic virus are, in this aspect at least, 2 different viruses. There were no apparent differences between RMV 1 and the virus used by Severin and Tompkins, thereby implying that they were not using the original radish mosaic virus, even though some of the symptom illustrations were taken directly from Tompkins' earlier paper.

All of the other results with RMV 1, such as the similarity of reactions on crucifer hosts to CBV, the inability to cause infection outside the Cruciferae, the high thermal inactivation point, and the failure to cross protect against CBRV, comply with the criteria for members of the cauliflower mosaic virus group. Further, purified preparations of RMV 1 and CBV show that each has spherical virus particles about 50 mμ in diameter and are indistinguishable in gross morphology. This evidence indicates that RMV 1 is a strain of the cauliflower mosaic virus. It seems probable that Severin and Tompkins used this virus rather than the virus originally described by Tompkins. Our failure to obtain short rigid rods with RMV 1 by Takahashi's described method leads us to believe either that his description did not deal with the cauliflower mosaic virus or that his inoculum consisted of 2 viruses, 1 of which (short, rigid rods) was not recovered from the frozen inoculum we received. The identity of the virus producing the short, rigid rods is in question. Our results do not completely clarify the picture but only point up the areas of confusion.

Sylvester's proposal of a separate group for the radish mosaic virus was based on: 1) the high dilution end-point and longevity *in vitro;* 2) the high thermal inactivation point; 3) transmission by *Myzus ornatus;* and 4) a short, rigid rod particle shape. The first 2 criteria are based on the virus described by

TABLE 2. Cross protection reactions of radish mosaic virus isolates against CAV and CBRV[a]

Radish isolate	Reaction against CAV	Reaction against CBRV
RMV 1	—[b]	—
RMV 2	+	+
RMV 3	—	—
RMV 4	+	+
RMV 5	+	+
RMV 6	+	+
WMV	+	+
CBV	—	—

[a] Viruses CAV and CBRV are known strains of turnip mosaic viruses, and CBV is a known strain of cauliflower virus 1.

[b] — indicates no cross protection; + indicates cross protection.

Tompkins, and the last 2 are taken from Severin and Tompkins', and Takahashi's reports. Since it is believed that 2 different viruses were involved in establishing these criteria, Sylvester's proposal cannot be justified.

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A SELECTIVE METHOD FOR ISOLATION AND MEASURING THE POPULATION OF PYTHIUM IN SOIL

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SUMMARY

A method has been described for the quantitative enumeration of the population of *Pythium* in soils. Martin's peptone-dextrose-rose bengal agar with 0.01 or 0.05% Terraclor (pentachloronitrobenzene) has been found to reduce greatly the number of contaminating molds in soil plates. Pimaricin, an antifungal antibiotic, used in concentrations of 0.001 to 0.002% in the same medium, completely eliminates other fungi from soil plates without adversely affecting the development of *Pythium*.

Introduction.—Methods for quantitative and qualitative study of soil-microflora have long been a subject of study by soil microbiologists. Many methods have been suggested as suitable for particular conditions and purposes. The vegetative nature of fungi and their widely different nutritional requirements make it difficult to get an accurate picture of their population in the soil, especially when only one particular medium or method is used. In spite of much work, the methods for detection, isolation, and quantitative estimation of specific soil-borne plant pathogenic fungi are less than satisfactory. Some are lengthy, others require special equipment and precautions, and most of them lack accuracy.

The dilution-plate and Warcup's soil-plate (37) techniques have been used widely as plating methods for the enumeration of total fungi in soil samples. Further improvements in these methods have been brought about by the development of better synthetic media (18, 20, 26, 33). Elimination of fungi from soil platings for accurate counting of bacteria and actinomycetes has been achieved by the use of antifungal substances (9, 10, 14, 29, 30), and similar attempts have been made for the elimination of bacteria and actinomycetes from soil platings to facilitate the enumeration of soil fungi (2, 10, 12, 20, 23, 36). The use of rose bengal, oxgall, and sodium propionate has made it possible to minimize the rate of growth of spreading fungi in soil platings, thus permitting better counts (26, 29, 33). Various methods also have been recommended for sampling, direct estimation, and isolation

of fungi from soil by the use of buried slides, tubes, etc. (5, 6, 7, 8, 24, 35, 39).

Relatively fewer reports concern attempts to measure the population of a particular fungus from soil samples. Here again, most of the methods were designed primarily for detection and isolation (4, 22, 38). Baiting with susceptible plant materials has been the most commonly used method for studying specific soil fungi causing plant diseases. Knaphus and Buchholtz (17) used baiting for measuring the population of *Pythium graminicolum* Subr. in the soil. Barton (3) has recently used this method for estimation of the population of *P. mamillatum* Meurs. in the soil. Klotz and DeWolfe (16) used lemon, oranges, and apples to trap and isolate *Phytophthora* spp. from infested soil and plant tissues. The bait-technique has also been used by Maloy and Alexander (19) for *Fusarium solani* f. *phaseoli* (Burk.) Snyd. & Hans. and *Thielaviopsis basicola* (Berk. & Br.) Ferr. and by Papavizas and Davey (27) for *Rhizoctonia solani* Kühn. McLaughlin (21) isolated and counted *Pythium* isolates by placing small quantities of soil beneath 2% water agar in petri dishes. Angell (1) attempted a soil-fractionation method for quantitative determination of *Pythium* sp. in soil particles. None of these methods provides a technique as simple and selective as those developed for enumerating bacteria and actinomycetes.

Selective media have been reported for the isolation of at least a few fungi. Russell (32) reported a medium, using *O*-phenyl phenol as inhibitor, in the selective isolation of basidiomycetes from wood pulp and from air in pulp mills. Schneider (34) reported that Acti-dione was inactive against *Graphium ulmi* and *Alternaria* sp., and could be used in the preparation of a selective medium for isolating these fungi. Nadakavukaren and Horner (25) used absolute alcohol in water agar as a selective medium for enumerating *Verticillium* microsclerotia in soil. While this manuscript was being written, Eckert and Tsao (11) and Hansen (13) have reported the use of nystatin and pimaricin for the selective isolation of *Phytophthora* spp. from plant tissues. These reports indicate that suitable chemicals, including antifungal antibiotics, may be helpful in formulating a medium selective for specific soil-borne plant pathogens.

The object of the present paper is to describe a procedure which inhibits or completely eliminates growth of most soil fungi, but facilitates the development of *Pythium* in soil platings. The method has been developed in a study on the physiology and behaviour of soil-borne plant pathogens as affected by different environmental conditions.

METHODS.—Sterilized infested and unsterilized artificially- and naturally-infested muck soils were used in these studies. *Pythium debaryanum*, isolated from corn seedlings, and another isolate of *Pythium* obtained from naturally-infested muck soil were used for pure culture studies on solid media and for infesting the soil. Most of the experiments used both the dilution-plate (1:10,000) and soil-plate techniques. The plates were incubated at room temperature (24-28°C). Counting of fungal colonies in soil platings was done first after 24 hours under a dissecting microscope and then on 2 successive days when the colonies had become visible and *Pythium* could be easily recognized. Agri-mycin (a combination of 15% streptomycin, 1.5% oxytetracycline, and 83.5% inert material) was used as antibacterial agent in all the media tried. Earlier studies had shown that this antibiotic did not affect the growth of *Pythium*, even at the rate of 150 mg/liter of medium.

A preliminary screening of several media was carried out to find the best one for further studies. These media were potato-dextrose agar, soil-extract agar, Martin's peptone-dextrose-rose bengal agar (20), Ohio Agr. Expt. Sta. agar (33), and Papavizas and Davey's dextrose-peptone-yeast extract agar (26). Several modifications of these media, made by varying the concentration of inhibitory substances, also were tested.

RESULTS.—The results of preliminary screening of media have shown that rose bengal, oxgall, and sodium propionate, used as inhibitory substances separately or in combinations, reduced the size of fungal colonies, including those of *Pythium*. Oxgall and sodium propionate were stronger inhibitors than rose bengal, especially for *Pythium*. However, none of these produced strong effects against *Rhizopus* sp., which could overgrow other colonies when present in the plate. On potato-dextrose and soil-extract agar the growth of *Pythium* was very rapid, even in the presence of inhibitors, and the colonies soon fused to make the counting difficult. The Ohio Agr. Expt. Sta. agar and dextrose-peptone-yeast extract agar reduced the size of *Pythium* colonies more than those of many other fungi, thus making it difficult to locate the fungus in the presence of large numbers of contaminants, especially species of *Rhizopus* and *Trichoderma*. The characteristic, easily-recognizable growth of *Pythium* on peptone-dextrose-rose bengal agar made this medium the one of choice, although *Rhizopus*, *Trichoderma*, and *Penicillium* developed faster than *Pythium*. The number of fungal colonies was not affected appreciably by the different media, but the media containing inhibitory substances facilitated more accurate counting. From the preliminary screening, it became apparent that irrespective of the medium used, the elimination of fungi growing faster than *Pythium* is essential for easy and accurate counting of this fungus in soil platings.

Terraclor, a commercial product whose active ingredient is pentachloronitrobenzene (PCNB), is known to inhibit the growth of many soil fungi such as *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, etc., but not that of *Pythium*, *Phytophthora*, and *Fusarium* (28). Porter (30) reported that pimaricin, a polyene antifungal antibiotic, was very effective in suppressing the growth of *Rhizopus* and *Penicillium* in soil platings for isolation of actinomycetes. Terraclor and pimaricin were

TABLE 1. Population of *Pythium* and other fungi in soil as determined by dilution-plate method using 4 media with varying concentrations of Terraclor and pimaricin (A-5283)*

Media and concentration of inhibitor	No. of colonies recovered from 100 mg soil (oven dry basis)				
		Unsterilized soil			
	Sterilized soil artificially reinfested with *Pythium*	Artificially infested		Naturally infested	
		Pythium	Other fungi	*Pythium*	Other fungi
Potato-dextrose agar (check)	1412	238	4047	46	910
PDA + 0.005% terraclor	1412	238	1190*	42	565
PDA + 0.01% terraclor	1165	238	478**	42	427*
PDA + 0.05% terraclor	1412	0	714**	46	261**
PDA + 0.0002% pimaricin	1165	238	2143	46	205**
PDA + 0.001% pimaricin	1412	476	714**	46	109**
PDA + 0.002% pimaricin	1412	238	238**	39	103**
Peptone-dextrose-rose bengal agar (check)	1165	238	4047	52	1055
PDRB + 0.005% terraclor	1412	476	3096	49	670
PDRB + 0.01% terraclor	1695	238	1667*	52	464*
PDRB + 0.05% terraclor	1695	476	967**	46	379*
PDRB + 0.0002% pimaricin	1412	476	1904*	58	349*
PDRB + 0.001% pimaricin	1695	476	238**	58	91**
PDRB + 0.002% pimaricin	1412	714	238**	55	24**
Ohio Agr. Expt. Sta. agar (check)	1412	238	4694	39	1152
OAESA + 0.005% terraclor	1165	238	967**	36	1062
OAESA + 0.01% terraclor	1165	238	714**	39	879
OAESA + 0.05% terraclor	847	476	476**	33	609*
OAESA + 0.0002% pimaricin	565	238	1667*	33	494**
OAESA + 0.001% pimaricin	282*	0	0	33	227**
OAESA + 0.002% pimaricin	282*	0	238**	24	109**
Dextrose-peptone-yeast extract agar (check)	1412	476	1904	36	1169
DPYEA + 0.005% terraclor	847*	0	714*	42	1085
DPYEA + 0.01% terraclor	847*	238	476*	39	806
DPYEA + 0.05% terraclor	282**	238	476*	33	582*
DPYEA + 0.0002% pimaricin	282**	0	1429	30	752*
DPYEA + 0.001% pimaricin	565**	0	238**	15*	115**
DPYEA + 0.002% pimaricin	282**	0	0	15*	76**

* The pimaricin (A–5283) was obtained through the courtesy of Dr. J. N. Porter of Biochemical Research Section, Lederle Laboratories, American Cyanamide Company, Pearl River, N. Y.

* The differences from treatment check are significant at 5% level.

** The differences from treatment check are significant at 1% level.

tried on the assumption that they might suppress the growth of other fungi in the plates without adversely affecting *Pythium*. Different concentrations of these 2 compounds were separately incorporated in the 4 basal media referred to earlier. Terraclor in water was added to the medium before sterilization, whereas pimaricin was added after the sterilized medium was cooled and ready for pouring into plates.

The results (Table 1) show that both Terraclor and pimaricin significantly reduced the numbers of fungi, except *Pythium*, in soil platings on all the 4 media. Terraclor was highly effective against *Rhizopus* and at concentrations of 0.01% and 0.05% no colonies of *Rhizopus* developed in any plate. Pimaricin was more effective than Terraclor in reducing the total number of contaminants. At concentrations of 0.001 and 0.002%, it completely eliminated the soil fungi except *Pythium* which was not affected in the number of its colonies or nature of its growth. The effect lasted for more than 3 days when the plates were incubated at 24-28°C. During this period, the colonies became well developed on peptone-dextrose-rose bengal agar. Both these compounds, however, were found to produce adverse effects on *Pythium* in media that also contained oxgall. At higher concentrations of Terraclor and pimaricin in Ohio Agr. Expt. Sta. agar and dextrose-peptone-yeast extract agar, there was a significant reduction in the number of *Pythium* colonies. The experiment was repeated several times with similar trend in results. On the basis of these findings, Martin's peptone-dextrose-rose bengal agar was selected as the most suitable medium to be used with pimaricin for studying the population of *Pythium* in the soil.

Table 2 shows further data regarding the effect of different concentrations of pimaricin on the development and frequency of appearance of *Pythium* in soil platings, on the relative frequency of other fungi, and also on the colony diameter in pure cultures. Apparently, a significantly greater population of *Pythium* was obtained with the elimination of other fungi. Further, no adverse effect on the growth of the fungus in pure culture was observed on peptone-dextrose-rose bengal agar containing different concentrations of the compound.

A comparative study of different methods of plating the soil was made using peptone-dextrose-rose bengal-

TABLE 2. Effect of different concentrations of pimaricin on radial growth of *Pythium* in pure culture and on the recovery of *Pythium* and other fungi from soil in soil platings

Concentration of pimaricin %	Radial growth in 48 hr[a] mm	No. of colonies recovered from 100 mg soil[b] (oven dry basis)	
		Pythium	Other fungi
A. None (check)	22.0	53	669
B. 0.0002	23.3	51	411**
C. 0.0005	22.3	65*	202**
D. 0.0007	23.0	60*	176**
E. 0.001	22.0	105**	8**
F. 0.002	21.0	99**	3**
G. 0.005	18.7	91**	0

[a] Based on 4 radial measurements from triplicate plates.
[b] Based on 6 replications of each treatment.
* Differences from check are significant at 5% level.
** Differences from check are significant at 1% level.

Agri-mycin agar with 0.001% pimaricin. The methods were: 1) soil-plate with (a) undiluted soil, or (b) soil diluted with wet or dry sterile soil; and 2) dilution-plate. Soil of the same type as the one plated was used as a diluent after it was well pulverized and autoclaved at 15 lb pressure for 2 hours. Mixing with dry, powdered soil was not very convenient and failed to give a good mixture. The mixing was done in small flasks by shaking for 1 minute and then rolling the flasks for 5 minutes. No aseptic precautions were thought necessary during mixing.

Use of the soil-plate method with undiluted soil involved least disturbance of the soil sample. Further, the elimination of other fungi by pimaricin permitted an accurate colony count even in platings of undiluted soil. Therefore, the results from platings of undiluted soil were taken as a check on other figures (Table 3). Although the differences in average counts obtained by plating the same soil by different methods were not statistically significant, the results were inconsistent at higher dilutions (1:20 or above with soil and 1:1,000 or above with water). Dilutions with soil up to 1:10 and in water up to 1:100 have given uniform counts of *Pythium* repeatedly, these being close to the values of the check. Since the competition from other fungi is eliminated by the use of pimaricin, the soil-plate method is definitely superior to dilution-plate technique where too much shaking and mixing is likely to break soil particles bound together by a single fungal colony, resulting in erroneous counts. Further, dilution of naturally-infested soil, where the population of the pathogen is low, may necessitate a large number of replications of each sample to get an accurate picture.

DISCUSSION.—Selective enumeration of *Pythium* in soil platings can be made easy and accurate by the use of suitable chemicals, including an antifungal antibiotic. Present studies have demonstrated the suitability of pentachloronitrobenzene and pimaricin for making selective enumeration of *Pythium* in soil platings by eliminating rapidly growing competitors. These studies, in part, support the findings of Eckert and Tsao (11) and Hansen (13), who demonstrated selective isolation of *Phytophthora* spp. from plant tissue by using pimaricin.

There is need for caution in selecting media to be used with such compounds. As has been stated, these compounds have adversely affected *Pythium* when used in media containing oxgall. The reason for this phenomenon was not investigated. Extension of the present work to find a suitable substitute for pimaricin has revealed that a crude preparation of phytoactin when added to peptone-dextrose-rose bengal agar at a very low concentration (0.5-1 ppm) eliminates the inhibitory effect of rose bengal and the fungus grows as fast as on potato-dextrose or soil extract agar. Randhawa and Hamner (31) have reported that a combination of growth regulators with different antibiotics reduced the inhibitory effect of the former. Gottleib, *et al.* (12) have recently reported sterols to antagonize the inhibitory action of filipin. While in the present studies the situation is somewhat different, there is a possibility that some type of chemical reaction does occur to prevent the development of *Pythium*. Since pimaricin is not yet easily available in the market, there is need for screening more fungicides and antibiotics, including polyenes like nystatin, on these lines. Fungal genera, species, and even strains within a species differ in their tolerance to fungicides and antibiotics. Therefore, there is every possibility that suitable compounds may be found for the selective enumeration of not only *Pythium* but other soil-borne plant pathogenic fungi.

When a selective medium is used, the method of plating out the soil sample poses no serious problem. The soil-plate method (37) and the dilution-plate method are both satisfactory provided that a relatively low

TABLE 3. Comparison of different methods of plating for counting of *Pythium* spp. in soil using peptone-dextrose-rose bengal agar with 0.001% pimaricin

Methods	No. of *Pythium* colonies recovered from 100 mg soil (oven dry basis)		
	Sterilized soil, artificially infested	Unsterilized soil	
		Artificially reinfested	Naturally infested
Soil-plate, undiluted soil (check)	143	158	56
Soil-plate, soil diluted with sterile wet soil:			
1:10	164*	172*	73*
1:20	328	431	73
1:60	0	259	0
Soil-plate, soil diluted with sterile dry soil:			
1:10	164*	144*	52*
1:20	344	316	52
1:60	0	0	0
Dilution plate:			
1:100	136*	103*	16*
1:1000	197	86	18
1:10000	328	0	0

* The values at these dilutions were reproducible and not significantly different from the check at 5% and 1% levels.

rate of dilution is used. The authors prefer the soil-plate method, since it involves least disturbance of the soil. The method described above also enables platings without rigid aseptic conditions. Since pimaricin rapidly deteriorates at high temperature and in the presence of moisture and sunlight, it should be used immediately after the suspension is prepared. The plates should be incubated at temperatures below 30°C.

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THE EFFECT OF SOIL MOISTURE UPON DEVELOPMENT OF POINSETTIA ROOT ROTS

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SUMMARY

Under controlled experimental conditions, soil moisture has a significant effect upon development of the poinsettia root rots caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr., *Rhizoctonia solani* Kühn, and *Pythium ultimum* Trow. *Thielaviopsis* caused appreciable damage at 36% of the moisture holding capacity (MHC), and disease increased as moisture increased up to about 70% MHC. Above this, an increase in moisture had no further effect. Both *Rhizoctonia* root and stem rots were most severe at moisture levels below 40% MHC, and disease severity decreased with increasing soil moisture. Above about 80% MHC *Rhizoctonia* caused little or no damage to poinsettia. At moisture levels of 30-40% MHC, *Pythium* caused only slight damage and disease severity was not greatly increased by increasing soil moisture to about 70% MHC, but above the latter moisture level *Pythium* root rot became a serious problem.

INTRODUCTION.—The root rots of poinsettia (*Euphorbia pulcherrima* Willd.), caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr., *Rhizoctonia solani* Kühn, and *Pythium ultimum* Trow, are by far the most important diseases associated with commercial poinsettia production under glass. The major damage caused by *R. solani* and *P. ultimum* generally occurs during the summer and early fall when the succulent rooted cuttings are young and are becoming established after transplanting from rooting beds. *T. basicola*, on the other hand, is apt to cause heavy losses during any part of the growing season, but is more often associated with root rot and defoliation of older plants from mid-November through December. All 3 of these pathogens may, however, occur in any combination as a complex. This greatly complicates the problem of control since the organisms involved are known to respond differently to fungicides and certain changes in environment. *Rhizoctonia* may be controlled effectively with Terraclor (pentochloronitrobenzene) and Captan [N-(trichloromethylthio)-4-cyclohexene-1, 2 dicarboximide] has sometimes given good control of *Pythium*. Where *Thielaviopsis* has been involved, however, none of the available fungicides tested has proved effective. Because of this, attention recently has been focused on the role of environment in the development of these diseases, as well as the disease complexes, in hope of obtaining information useful in achieving more effective control.

It was previously shown that each of the poinsettia root rots is strongly influenced by soil temperature (4). *Thielaviopsis* root rot is most severe from 13-26°C, while *Pythium* root rot is most severe from 13-21°C. Both diseases, and particularly the latter, are restricted at higher temperatures. In contrast, *Rhizoctonia* root and stem rots are most severe at 30°C, the highest temperature tested, but cause considerable damage at temperatures as low as 21°C. At still lower temperatures the *Rhizoctonia* diseases are suppressed. Thus, at any temperature at which poinsettias can be grown commercially they are susceptible to attack by 1 or more of these root rot pathogens. To increase our knowledge of the role of environment in development of the poinsettia root rots, the present investigations on the influence of soil moisture were undertaken. In these studies an effort was made to hold soil temperature in a range favorable for development of the disease under study while the soil moisture effects were being determined. A preliminary report of this work has already appeared (2).

LITERATURE REVIEW.—As early as 1919, Johnson and Hartman (10) demonstrated that a high soil moisture is not requisite for damaging infection of tobacco by *Thielaviopsis*. They found that *Thielaviopsis* root rot of tobacco was capable of developing in plants grown in relatively dry soil (25% moisture holding capacity-MHC); increasing the moisture content of the soil to 75% of its MHC did not materially increase root rot, but saturated soils were more favorable for disease development than unsaturated ones. Later, Sattler (15), working at 3 moisture levels (30, 60, and 90%-MHC) with *Thielaviopsis* root rots of bean and tobacco, reported an increase in severity of both diseases as soil moisture was increased from 30-90% MHC. Sattler did, however, obtain moderate infection at 30% MHC, the lowest soil moisture tested. Keller and Shanks (12) reported that *Thielaviopsis* root rot induced defoliation of poinsettias several weeks sooner at high soil moistures than at low and normal moisture levels. They also presented data which suggests that *Pythium* root rot causes more damage to poinsettia under conditions of high soil moisture than at lower levels.

Flor (7) has shown that *Pythium* is most damaging to corn at moisture levels of 70-90% MHC, but in his experiments he did not obtain seed germination at levels above 90% MHC. *Pythium* caused no damage

at 20 and 30% MHC; but when the plants were held at moistures above 50% MHC, *Pythium*-infected plants were smaller than healthy ones. Roth and Riker (14) observed that *Pythium* damping-off of red pine seedlings increased linearly as soil moisture was increased from 15-100% MHC, whereas *Rhizoctonia* damping-off was greatest in drier soils (10-70% MHC). In the case of *Rhizoctonia* damping-off high air humidity increased losses irrespective of soil moisture. Wright (17), working at moisture levels of 50 and 95% MHC, observed *Pythium* damping-off of American elm, Siberian elm, black locust, and desertwillow seedlings to be more severe at the higher soil moisture level. *Rhizoctonia* also caused most damage at the lower moisture level in all species except desertwillow.

Abdel-Salem (1) found a variable reaction among isolates of *R. solani* in relation to soil moisture and damping-off of lettuce. An isolate from lettuce caused most severe damage at a high soil moisture level (80-90% MHC), whereas a tomato isolate caused most damage at a low level (25-35% MHC). Blair (5) demonstrated that *R. solani* grows best in dry soils, but an increase in moisture had little effect when the fungus was grown in sand. Because of this, he suggested that soil moisture decreases the growth of *Rhizoctonia* by decreasing the oxygen tension in the soil.

Materials and Methods.—The fungus isolates used in these experiments were all from poinsettia. Poinsettia cuttings (var. Barbara Ecke Supreme) were selected from vigorously growing stock plants. They were rooted under intermittent mist in a rooting tray containing vermiculite and maintained at about 26°C by an electric heating cable. The rooting period was 16-21 days.

The principal problem posed in these experiments was that of maintaining relatively constant soil moisture levels throughout an experimental period of about 3 weeks. It was decided at the start not to use the common method of weighing containers daily and bringing them to the desired weight by adding water, since moisture distribution in soil, when added to containers, is quite uneven (9). For these experiments, it was desired to have the moisture distribution throughout the soil mass as uniform as possible, because an uneven moisture distribution may give an erroneous picture of disease development.

Moinat (13) has used an auto-irrigator which depends upon the capillary rise of water through sand to supply water for plants growing in soil above a porous plate embedded in the surface of the sand. He was able to obtain high, moderate, and low moisture supply for plants of moderate size by varying the depth or textural grade of sand above a water source. Roth and Riker (14) used metal cylinders of various heights filled with soil and placed over a constant water table to obtain different moisture levels in the surface layers of the soil. This type of moisture control is perhaps satisfactory for damping-off studies where the pathogens would be active mainly near and at the soil surface. For the purposes of root rot investigations, however, the results may be complicated by the fact that the roots of the plants will continue to grow down through the soil, and the moisture content of the soil increases with vertical depth in such a system.

In the present work, moisture control was achieved by varying the depth of a medium (⅓ vermiculite, ⅔ builders' sand by volume) above a constant water table. Metal greenhouse benches 4 ft x 7 ft x 6 in. served as the basic units for the moisture control beds. About 1 in. of coarse gravel was placed in the bottom of these benches and was overlayed with nylon screen. A constant water table was maintained with automatic float valves so that the water level was about 0.25 in. above the screen. The sand-vermiculite mixture was then placed in the beds, care being taken to get uniform compaction. The depth of medium was adjusted in height by trial and error until the desired moisture levels in the surface layers were obtained. Where a depth greater then 6 in. was required, the sides of the moisture beds were extended with wooden racks to accommodate the necessary depth of sand-vermiculite medium. Since moisture varies with vertical depth in such a system, and capillary movement of water in soil is quite slow, particularly in dry soil, 2.5 in. clay pots were used to contain the soil in which the plants were grown. These small pots were placed in the surface layers of the sand-vermiculite medium in the beds so that their rims were flush with the surface. The use of these pots in the surface limited the root systems of the plants to a zone not greater than 2.3 in. below the surface, thus reducing the possibility of any portion of the root system being exposed to moisture greatly different from other portions. Also, the use of small pots reduced the length of the diffusion path from the pot wall to the center of the pots, making it easier to maintain more uniform moisture conditions throughout the soil. A diagram of 1 of these beds is given in Fig. 1.

The soil mixture consisted of ⅓ loam, ⅓ sand, and ⅓ peat by volume. This mixture had a pH of 7.0±0.3 and held about 56% moisture, expressed on a dry weight basis, when saturated. The soil mixture and the moisture beds were steam sterilized before each experiment.

Moisture levels near 30, 50, 70, and 90% MHC were obtained by using a medium depth of 12, 8, 5, and 3 in., respectively, above the water table. Daily moisture levels were followed by using an impedance comparator, and taking electrical readings approximately 1.5 in. below the sand-vermiculite surface. Soil moisture (impedance) readings were made with an impedance comparator (Soil Moisture Indicator) manufactured by the VenTender System, Chagrin Falls, Ohio. The readings were taken in terms of units of needle deflections (meter readings 0-100) on a 3-volt A-C meter. These readings were then correlated with dry weight determinations of the soil within the pots.

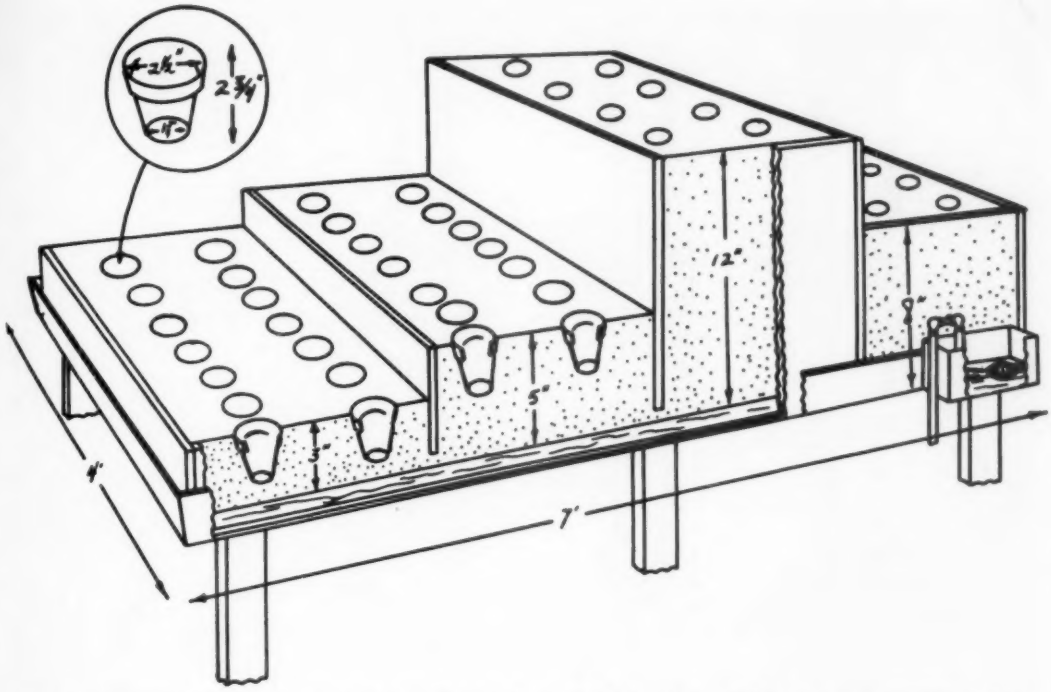

Fig. 1. Diagram showing a cross-sectional view of a metal greenhouse bench modified for soil moisture control. Moisture levels near 90, 70, 50, and 30% MHC in pots of soil were obtained when a sand-vermiculite medium (1:1 by volume) was placed over a constant water table at respective depths of 3, 5, 8, and 12 in.

Dry weight determinations of soil moisture were made for each soil moisture level in each experiment.

Three pots of soil were selected at random from each moisture level and used to estimate the moisture content of all the pots at the particular moisture level sampled. These 3 dry weight values were averaged, and the mean values of all soil samples taken during the experiment were used to compute the linear regression of soil moisture on the electrical readings. Twenty-four samples, each consisting of 3 pots, were taken. The duration of experiments varied from 11-23 days; usually the samples were taken at the end of the experiments, though some were taken as early as the 4th day after inoculation. The linear regression curve was used to estimate moisture levels in the beds from the electrical readings.

In essence, the moisture control beds depended on 3 capillary systems—the sand-vermiculite medium, the clay pots, and the soil within the pots. A "constant" moisture level was reached faster when the entire beds were saturated after the potted plants were placed in the sand-vermiculite medium, then drained, and the water table re-established. After the beds were saturated, drained, and the water table re-established, they were allowed to stand until they approached an "equilibrium." This usually required about a week or so depending upon the temperature and relative humidity. After this the soil, in which the plants were growing, was infested, using a hypodermic syringe to inject the inoculum into the soil in 3 areas in each pot, about 1 in. below the surface. The inoculum for these experiments was grown as previously described (4), then fragmented in a Waring Blendor, and diluted with water so that each pot received 0.1 of a petri plate culture in 5 ml of suspension.

Data on root rot severity were taken, on the average, 14 days after soil infestation with *Rhizoctonia* and *Pythium;* and 20 days after soil infestation with *Thielaviopsis.* The root systems were washed free of soil and rated visually for disease severity according to a 0-5 index (4). Fresh root weights of diseased and healthy plants were also taken.

Results.—In a preliminary study, root growth of poinsettia was retarded at soil moisture levels below about 30 or above 87% MHC, but was little affected at soil moistures between these values over the relative short experimental periods employed. Root growth was determined by fresh weight at the end of the experiments. A total of 55 check plants (uninoculated) were grown at each of 4 moisture levels, near 30, 50, 70, and 85% MHC, and the respective average root weights at these moisture levels were 4.2, 4.0, 3.8, and 4.0 g.

Thielaviopsis.—Two experiments were performed to determine the influence of soil moisture upon the development of *Thielaviopsis* root rot of poinsettia. In

Fig. 2. Root systems of **(A)** healthy, and **(B)** *Thielaviopsis*-infected poinsettias after 21 days growth in soil at the following moisture levels (left to right): 38, 49, 60, 80% MHC.

the 1st experiment, plants were inoculated on August 12, 1958, and data were taken on August 31. Analysis of soil samples at the end of the experiment showed a moisture content of 36, 60, 70, and 87% MHC, and the respective average root rot index readings were 2.3, 3.4, 4.1, and 3.1. There were 12 inoculated and 12 uninoculated plants at each moisture level. The root systems of the uninoculated plants remained healthy in all cases. The soil temperature was near 22°C.

The soil moisture control during this experiment, based on estimates derived from the electrical readings, was very good. The maximum estimated moisture change did not exceed 7% MHC from the time the plants were inoculated until data were taken.

The 2nd experiment with *Thielaviopsis* was carried out in September, 1958. Soil temperature was about 20°C during this experiment. Analysis of soil samples at the end of the experiment revealed a moisture content of 38, 49, 60, and 80% MHC. The respective average root rot indices at these moisture levels were 2.4, 3.0, 4.1, and 4.4 (Fig. 2). There were 24 inoculated and 12 uninoculated plants at each moisture level. As before, the uninoculated plants remained healthy.

At the lowest moisture level tested, *T. basicola* was quite destructive; 25-50% of all roots of inoculated plants were destroyed within 3 weeks after inoculation. Disease severity increased with increasing soil moisture up to 60-70% MHC; at this moisture level, 50-100% of the roots of inoculated plants were destroyed in the experimental periods. At moisture levels between 60-70% MHC and 36-38% MHC, disease development was intermediate. Root rot severity was not significantly altered by increasing soil moisture from 60-70% MHC to 80-87% MHC.

Pythium.—Three experiments were conducted with *Pythium*. In the 1st, plants were inoculated July 21, 1958, and the data were taken August 6. In this experiment soil moisture averaged 31, 41, 70, and 87% MHC, and the respective average root rot index readings were 0.0, 0.0, 0.3, and 3.0. Moisture variation was considerable in this experiment, especially at the 2 lower moisture levels. The lowest moisture level initially was 50% MHC, but was 16% MHC at the end of the experiment. The 2nd lowest moisture level started at 47% MHC, and ended at 32.9% MHC. At the 3rd moisture level, the moisture fell from 73% MHC at the start, to 61% MHC by the end of the experiment; at the highest level, the moisture content varied 84-88% MHC.

The moisture variation in this experiment may be attributed in part to at least 3 different things: 1) the

plants were inoculated only 2 days after they had been placed in the moisture beds and thus the soil had not attained moisture equilibrium; 2) the experiment was conducted in July and August when the soil temperature averaged about 24°C, while the air temperature was considerably higher; 3) the sand-vermiculite medium in the bed was relatively new and was not as compact as it should have been. All of these factors acting together may account in part for the observed moisture changes.

Since it is known that high temperature tends to reduce the severity of *Pythium* root rot of poinsettia (4), the other 2 experiments were not carried out until November. The 2nd experiment was started November 7 and ran through November 22. The soil moisture sample values at the end of this experiment were 29, 52, 65, and 88% MHC, and the respective average root rot index readings were 2.0, 2.8, 3.1, and 3.8. The soil temperature was maintained at about 17°C. Moisture control during this experiment appeared to be excellent; the maximum estimated variation was 3% MHC. In this experiment 9 inoculated and 5 uninoculated plants were employed at each moisture level.

The 3rd *Pythium* root rot experiment was started November 24 and ended December 12. The moisture sample values at the end of this experiment were 35, 48, 67, and 81% MHC, and the respective average root rot index values were 1.5, 1.9, 2.2, and 3.5. These results were very similar to those in the 2nd experiment. Soil temperature was held at about 16°C and the maximum variation in soil moisture was an estimated 5% MHC.

Pythium root rot was only slight at soil moisture of 30-40% MHC and was only moderately increased by increasing soil moisture up to 50-65% MHC. Within the latter moisture range, 25-50% of the roots of inoculated plants were destroyed on the average in about 2 weeks. When soil moisture was increased from this level, an abrupt increase in disease severity was detected in all experiments, and an average of about 75% of all roots of inoculated plants was destroyed.

Rhizoctonia.—In 3 *Rhizoctonia* experiments, root and stem rots were rated for severity separately; however, it was found that both of these rots were influenced similarly by soil moisture. The 1st *Rhizoctonia* experiment was carried out at the same time and in the same beds with the 1st *Pythium* experiment, thus the soil temperature (24°C) and moisture variations were the same. In this experiment 9 inoculated and 9 uninoculated plants were employed at each of the 4 moisture levels, which averaged 31, 41, 70, and 87% MHC. The respective average root rot indices were 0.6, 0.3, 0.3, and 0.0, while the stem rot indices were 0.9, 1.8, 0.8, and 0.0. Since the degree of infection in this experiment was so slight, very little weight should be attached to it.

Because of the low virulence of the *Rhizoctonia* in the 1st experiment, a reisolate from an infected plant was built up in an effort to increase virulence for the 2nd experiment. The 2nd experiment was carried out from August 12-31, 1958. This experiment was carried out simultaneously with the 1st *Thielaviopsis* experiment, with the same soil temperature (22°C) and moisture variation. The same uninoculated plants served as controls. In this experiment, *Rhizoctonia* root rot values were 3.1, 2.0, 0.9, and 1.3, while stem rot index values were 3.4, 2.0, 1.1, and 0.2. These correspond respectively to soil sample moisture values of 36, 60, 70, and 87% MHC.

The fungus was again isolated from diseased plants and inoculum built up in an effort to further increase virulence. Thus, the inoculum for the 3rd experiment was *Rhizoctonia* which had been passed through the host twice within a period of about 1 month. The fungus was exceedingly virulent and many of the plants at the lowest soil moisture level had toppled over only 1 week after inoculation due to severe stem infection. Data were collected 10 days after inoculation in an effort to catch the experiment at a point where the maximum differences could be detected. Soil temperature was maintained at about 22°C over the experimental period.

Soil moisture contents at the end of this experiment were 29, 45, 60, and 78% MHC. The corresponding disease index values for the moisture levels were 2.9, 1.8, 1.4, and 0.7; and 3.3, 2.3, 1.8, and 0.0, for root and stem rots, respectively. There were 19 inoculated and 10 uninoculated plants at each moisture level. All uninoculated plants were healthy at the end of the experiment.

Evidently, soil moisture markedly influenced the development of the *Rhizoctonia* root and stem rots, both rots being affected similarly by different moisture levels. At the lowest moisture levels tested, disease was most severe. There appeared to be an almost linear inverse relationship between disease development and soil moisture content between 36-80% MHC. At the latter moisture level, *Rhizoctonia* caused virtually no damage to poinsettia in these experiments, particularly with respect to stem rot; at the low moisture level, plants were sometimes rendered worthless within a 7-day period after inoculation, depending upon the virulence of the inoculum.

Symptoms of the above root rots of poinsettia have been described previously (4). In the present work, however, another characteristic feature of *Rhizoctonia* infection was noted, particularly in the drier soils. Superficial mycelia of the fungus to which soil particles adhered were readily observed when infected plants were removed from the pots and the soil gently shaken from the root systems. This particular characteristic may facilitate field identification of the disease.

The results of all of the above experiments are summarized graphically in Fig. 3 and the statistical significance of the data from each experiment is summarized in Table 1.

Discussion.—The preceding results demonstrate that soil moisture has a significant effect upon the development of each of the poinsettia root rots and

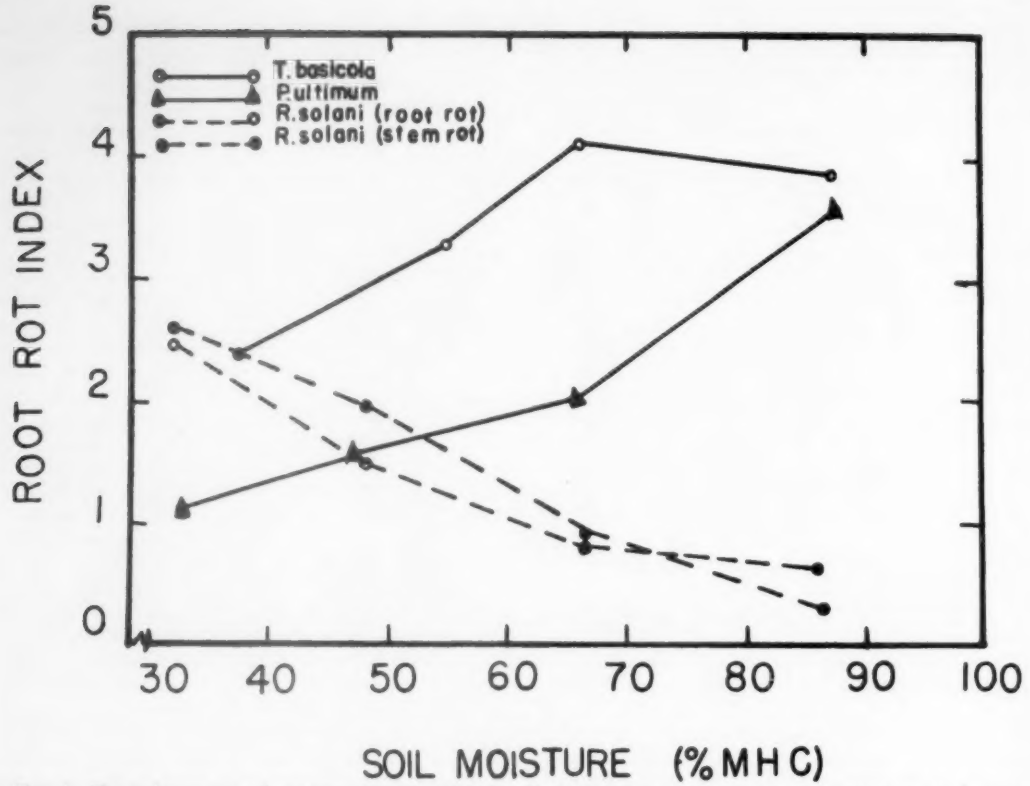

Fig. 3. The influence of soil moisture upon development of the poinsettia root rots caused by *Thielaviopsis basicola*, *Pythium ultimum*, and *Rhizoctonia solani*. Root weight of healthy poinsettia plants was relatively unaffected by these different moisture levels over the short experimental periods employed.

that damage caused by *Rhizoctonia* and *Pythium* can be reduced greatly by manipulation of soil moisture. *Thielaviopsis* root rot, however, although significantly reduced by low soil moistures, is serious over a very wide moisture range. Furthermore, while reduction of the *Rhizoctonia* root and stem rots by the use of high soil moisture and *Pythium* root rot by low soil moisture was good, it was not complete. Reduction of both *Rhizoctonia* and *Pythium* root rots by soil moisture manipulation would not be possible if both organisms were present.

The above results may be interpreted as indicating an indirect effect of soil moisture; that is, soil moisture may affect soil aeration, this in turn playing a major role in the development of the diseases caused by *Pythium* and *Rhizoctonia*. *Pythium*, a phycomycete, should be well adapted to a semi-aquatic environment, with resultant low oxygen tensions, while *Rhizoctonia* is favored by high oxygen and low carbon dioxide tension (5, 6). Also, since the results indicated that normal root growth was not affected to any great extent by soil moisture over the range employed, the influence of soil moisture upon disease development, direct or indirect, may be more closely associated with the pathogens than the host. More work on this point is needed.

In a recent paper, Hawker, *et al.* (8) indentified a vesicular-arbuscular endophytic mycorrhizal fungus, associated with roots of several plant species, as *P. ultimum*. It is conceivable that this fungus enters a mycorrhizal relationship with plant roots and becomes pathogenic when conditions in some way become unfavorable for the host. If this is the case, it may explain why *P. ultimum* causes damage mostly under conditions of high soil moisture and low soil temperatures, which are unfavorable for poinsettia growth, even though fungus growth in culture is favored by high temperature. This point at present is mere speculation, but it is perhaps worthy of future investigation.

The contrasting behavior of *Pythium* and *Rhizoctonia* was evident in the temperature study (4), where *Pythium* was found to cause severe infection only at low soil temperatures and *Rhizoctonia* only at relatively high temperatures, and again in the soil moisture studies which showed *Pythium* to be favored by wet soil and *Rhizoctonia* by dry soils. *Thielaviopsis* on the other hand is damaging over a relatively wide range of both of these factors.

Keller (11) has reported excellent control of *Thielaviopsis* root rot of poinsettia by using soil with a low

TABLE 1. The influence of soil moisture upon development of the poinsettia root rots

Disease	Experiment	Disease indices[a,b] at various moisture levels[c]			
Thielaviopsis root rot	A	2.3(36)	3.1(87)	3.4(60)	4.1(70)
	B	2.4(38)	3.0(49)	4.1(60)	4.4(80)
Pythium root rot	A	0.0(31)	0.0(41)	0.3(70)	3.0(87)
	B	2.0(29)	2.8(52)	3.1(65)	3.8(88)
	C	1.5(35)	1.9(48)	2.2(67)	3.5(81)
Rhizoctonia root rot & stem rot	A	0.6(31)	0.3(41)	0.3(70)	0.0(87)
		0.9	1.8	0.8	0.0
	B	3.1(36)	2.0(60)	1.3(87)	0.9(70)
		3.4	2.0	1.1	0.2
	C	2.9(29)	1.8(45)	1.4(60)	0.7(78)
		3.3	2.3	1.8	0.0

[a] The disease index values given represent the mean disease index of all inoculated plants grown at a given moisture level.

[b] According to Duncan's new multiple range test, all disease index values which are not underlined by the same line are significantly different from each other at the 5% level.

[c] The figures in parentheses indicate the average moisture content of the soil (% MHC) in which the plants were grown.

pH (pH 4.6-4.9). A variation of greenhouse temperature (50-70°F) did not seem to alter the pH effect on disease control. Shanks and Link (16) felt that maintenance of a low soil pH was the only alteration of environment which offers a practical control of this disease. More recently, Bateman (3) obtained good control of this disease by maintenance of a low soil pH (below pH 5.9), even in the presence of low soil temperature and high soil moisture, although both of the latter conditions are known to favor disease development. It has been shown that low soil temperature will control *Rhizoctonia* root and stem rots of poinsettia (4), low soil moisture will markedly reduce damage caused by *Pythium*, and *Thielaviopsis* root rot is greatly retarded by low soil pH (3, 11). It therefore appears that a combination of low soil temperature, low soil moisture, and low pH should result in minimum damage from the poinsettia root rots regardless of the pathogen or pathogens present. Further work will soon be reported in which the pathogens and various soil temperature, moisture, and pH levels were used in factorial combinations in an effort to determine more precisely the combination of environmental factors which will result in minimum disease, regardless of the combination of pathogens present.

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BOOK LISTING

ICONES FUNGORUM BAMBUSICOLORUM JAPONICORUM. Iwao Hino. 1961. The Fuji Bamboo Garden, Nagahara, Gotemba City, Shzuoka, Japan. 338 p.

The first part of this interesting paper-bound book describes (in English) properties, cultivation, and utilization of bamboo. The second, and largest, part of the book is made up of descriptions (in Latin) of parasitic fungi on bamboo in Japan. Each of nearly 175 descriptions is accompanied by drawings (by Ken Katumoto) illustrating spore forms and fruiting bodies. The third part describes (mostly in Japanese) types of bamboo prized for ornamental use because of various markings, some due to fungi.

SEED TRANSMISSION OF BEAN MOSAIC VIRUSES

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SUMMARY

Seed transmission of bean virus (BV1) and New York 15 virus (NY15), through either the pollen or ovules of infected plants, was obtained in high percentage by crossing diseased and healthy plants. The F_1 progeny resulting from pollination of resistant plants with infected pollen from susceptible plants contained a large number of infected plants when resistance was recessive, and no infected plants when resistance was dominant. Pollen usually transmitted virus to a larger number of progeny plants than ovules. BV1 and its strain, NY15, were similar in pollen and seed transmission and distribution of the virus in floral parts. Percentage of seed transmission of BV1 was not affected by growing infected mother plants at different temperatures. Bean virus 2 (BV2) was not seed-transmitted in any of the tests, nor was it recovered from anthers or embryos. Extracts from embryos and seed coats did not appear to inhibit or inactivate the viruses.

INTRODUCTION.—According to numerous reports, common bean mosaic virus and its related strain, the New York 15 virus, are commonly seed-transmitted. In contrast, most studies indicate yellow bean mosaic virus and its many related strains are rarely, if ever, seed-transmitted. This paper compares the viruses as to the tissues they infect and some factors influencing percentage of seed transmission. These viruses are hereinafter referred to as BV1 (bean virus 1, or common bean mosaic virus), BV2 (bean virus 2, or yellow bean mosaic virus), and NY15 (New York 15 virus) for the Burkholder strain of BV1.

REVIEW OF LITERATURE.—1) *Bean virus 1.*—Seed transmission of BV1 was first reported by Reddick and Stewart (18), who observed a great difference in percentage of virus transmission in seed from infected plants. Burkholder and Muller (4) reported that about 50% of the seed from diseased plants produced infected plants. Pierce and Hungerford (17) reported that seed transmission was usually about 33%, and that plants infected in early stages of growth produced seeds with somewhat higher transmission (48%). This was confirmed by Fajardo (10), who found a close positive correlation between earliness of infection and percentage of seed transmission. Nelson (14) attempted to explain seed transmission by only a portion of the seeds in a pod on an anatomical basis, postulating that virus may be present in only certain tissues of diseased plants and that the embryo is infected by contact with these tissues. Differences between several bean varieties in proportion of seed infection were reported by Smith and Hewitt (20), who found percentage of seed transmission higher in severely diseased varieties than in mildly diseased ones. Reddick and Stewart (18) suggested that pollen of infected plants may carry the virus to progeny of healthy ones. Frandsen (11) was able to demonstrate pollen transmission of BV1, but not BV2. Nelson and Down (15) reported transmission by both ovules and pollen.

2) *New York 15 virus.*—NY15 virus was first reported from New York (19), and later from Southern Idaho (8) when some varieties usually resistant to BV1 became infected. The virus was reported to be seed-transmitted, but no information has been published on factors affecting seed transmission. Cross-protection tests (12, 13, 22) have indicated that BV1, NY15, and several strains of BV2 are interrelated.

3) *Bean virus 2.*—BV2 was first described in 1934 (16). Since then a number of strains have been differentiated on the basis of symptoms on various hosts. The virus has a wider host range than BV1 or NY15, is not seed-borne, but is transmitted mechanically and by aphids. In 1955 Snow (21) isolated from the gladiolus variety Chamouny a virus that was 100% seed-borne in Bountiful beans, but on transfer from bean to bean seed transmission declined to 50%. The virus resembled BV1 in being seed-borne, and BV2 in its ability to infect Alsike clover and the Top Crop variety of bean. It did not, however, attack *Vicia faba,* which is usually susceptible to strains of BV2. Another virus shown to be a strain of BV2 by cross-protection tests was reported (6) seed-borne in lupines; transmission in beans was not tested.

MATERIALS AND METHODS.—1) *Sources of inoculum and bean varieties.*—BV1 was obtained from infected seed of Pinto bean (*Phaseolus vulgaris* L.) grown in the Salinas Valley, Calif.; NY15 from infected seed of Pinto U.I. 72 grown at Twin Falls, Idaho; and BV2 from infected plants of the Sutter Pink variety in the Agronomy plots at the California Experiment Station, Davis. Inoculum was made by grinding infected tissue in a mortar containing 0.05*M* phosphate buffer, pH 7. Leaves were inoculated by dusting with Carborundum and rubbing with the pestle used to grind the inoculum. Initial stocks of bean varieties were supplied by the Department of Agronomy at Davis. Virus-free seed of each variety was increased in the greenhouse from virus-free plants.

2) *Identification of virus strains.*—The identities of the virus collections were confirmed by host reac-

TABLE 1. Identification of BV1, BV2, and NY15 by means of differential reaction on several hosts

Host plants	Viruses		
	BV1	BV2	NY15
Alsike clover, *Trifolium hybridum* L.	—[a]	+[a]	—
Baby lima bean, *Phaseolus lunatus* L.	—	+	—
Bean, *P. vulgaris* L.			
Blue Lake	+	+	+
Bountiful	+	+	+
Great Northern 16	—	+	—
Idaho Refugee	—	+	—
Pinto U. I. 72	—	+	—
Pinto U. I. 111	—	+	+
Red Kidney	+	+	+
Small White 38	+	+	+
Sutter Pink	+	+	+
Cowpea, *Vigna sinensis* (L.) Endl.	—	+	—
Red Clover, *Trifolium pratense* L.	—	+	—
Horsebean, *Vicia faba* L.	—	+	—
Pea, *Pisum sativum* L.	—	+	—

[a] (—) indicates no infection; (+) indicates that infection resulted after inoculation.

tions (Table 1); by their inability to infect *Nicotiana glutinosa* L., *N. rustica* L., *N. tabacum* L., tomato (*Lycopersicon esculentum* L. var. Pearson), alfalfa (*Medicago sativa* L.), and celery (*Apium graveolens* L.); and by their physical properties.

Under field conditions, the BV2-infected plants showed mild yellow mottling and were branched more than normal plants, internodes were shorter, and yield of pods was smaller. In most bean varieties inoculated in the greenhouse, BV2 produced necrosis along the veins of primary leaves, necrosis along stems and petioles, and finally top necrosis. On 2 varieties, Sutter Pink and California Pinto, a large number of necrotic, coalescing local lesions developed on the trifoliate leaves, causing them to wilt and die. BV1 and NY15 caused typical green mottling, puckering, and some deformation of leaves of susceptible varieties.

RESULTS.—1) *Frequency of tranmission by different varieties and through pollen and ovules.*—Tests were made to determine differences among 3 varieties used in these studies in ability to transmit BV1 and NY15.

Seed of each of 3 bean varieties—California Pinto Sutter Pink, and Small White—was obtained from about 25 greenhouse-grown plants infected in the 2-leaf stage with 1 of the 2 viruses. The seeds were planted in 6-in. pots in the greenhouse, and counts of virus-infected seedlings were made about 3 weeks after emergence.

There are several reports (11, 15, 18) on pollen transmission of BV1, but there is only 1 report (15) that the virus is also transmitted by passage through ovules. Tests were therefore conducted to compare the frequency of ovule and pollen transmission.

Crosses were made between Small White and Sutter Pink involving various combinations of male and female, with 1 parent infected with BV1 or NY15. The seeds were planted in 6-in. pots in the greenhouse, and counts of virus-infected seedlings were made about 3 weeks after emergence.

The results (Table 2) indicate that BV1 was transmitted either through pollen or ovules of infected plants of both varieties. In 2 tests NY15 was transmitted through pollen in about the same percentage as BV1. When Small White was the female parent the percentage of ovule transmission, as well as pollen transmission by both Small White and Sutter Pink, was low (21% or lower). In contrast, when Sutter Pink was the female parent the percentage of ovule transmission and pollen transmission by both varieties was high (42% or higher). These differences are highly significant (χ^2). The results also show that BV1 and NY15 were transmitted with about equal frequency by seed from self-pollinated-infected plants, and that both Sutter Pink and California Pinto transmitted the viruses about twice as frequently as Small White. The percentage of transmission of BV1 by seed from Sutter Pink was significantly less (χ^2) from self-pollinated-infected plants than from artificial crosses in which only 1 parent was infected. This result was unexpected and no explanation for it can be offered.

In the same experiment, young plants of the variety Red Kidney were inoculated with BV2 and grown to maturity. None of several hundred seeds from these systemically-infected plants produced virus-infected plants.

2) *Seed transmission of BV1 and NY15 by resistant parents.*—Nelson and Down (15) reported that resistant female parents (var. Robust) receiving pollen from infected plants produced infected progeny. Ali (1) showed that the F_1 progenies of crosses of resistant and susceptible varieties of bean are either susceptible or resistant to BV1, depending on the

TABLE 2. Percentage of diseased plants of F_1 progenies of selfed diseased (BV1 or NY15) plants and from crosses between diseased (BV1 or NY15) and healthy plants

Cross [a]	No. of plants	% of transmission
SW x SW (BV1)	25	20
SW x SP (BV1)	34	18
SW x SP (NY15)	14	21
SW (BV1) x SW	41	8
SW (BV1) Self-pollinated	437	21
SW (NY15) Self-pollinated	124	23
SP x SW (BV1)	17	70
SP x SW (NY15)	25	60
SP x SP (BV1)	17	76
SP (BV1) x SW	27	67
SP (BV1) x SW (BV1)	21	86
SP (BV1) x SP	23	78
SP (BV1) Self-pollinated	456	42
SP (NY15) Self-pollinated	155	44
CP (BV1) Self-pollinated	482	45
CP (NY15) Self-pollinated	117	43

[a] Small White (SW), Sutter Pink (SP), and California Pinto (CP). The first-listed member of the cross was the female parent.

TABLE 3. Percentage of infected individuals in the F_1 progeny from reciprocal or single crosses of susceptible-infected (BV1) plants and uninfected plants of resistant varieties

Cross [a]	No. of plants	% of transmission
SP (BV1) x GN16	12	25.0
GN16 x SP (BV1)	54	48.0
P (BV1) x GN16	46	61.0
GN16 x P (BV1)	19	86.0
SP (BV1) x P111	28	35.0
P111 x SP (BV1)	32	83.0
SP (BV1) x GN123	20	10.0
GN123 x SP (BV1)	88	23.0
RM34 x P (BV1)	13	00.0
RM34 x SW (BV1)	15	00.0
IR x P (BV1)	54	00.0

[a] Susceptible varieties—Sutter Pink (SP), Pinto (P), and Small White (SW); resistant varieties—Great Northern (GN), Red Mexican (RM), and Idaho Refugee (IR). The first-listed member of the cross was the female parent.

source of resistance. Resistance derived from the Corbett Refugee variety, as in Idaho Refugee, is dominant, whereas resistance derived from Great Northern, as in Pinto U.I. 72 and 111, is recessive. Ali reported that inheritance of the 2 types of resistance is controlled by the interaction of 2 alleles, A*a* and I*i*. The genotype of Idaho Refugee was represented by AAII, in which an inhibitor gene, I, prevented the expression of A, a gene for susceptibility. In contrast, the genotype of Great Northern was represented by *aaii*, wherein *a* represents a recessive gene for resistance.

Crosses were made between recessive-type resistant varieties and susceptible varieties in which the susceptible member of the cross was infected and in reciprocal crosses served as either the male or female parent. Also, crosses were made in which susceptible-infected varieties served as the male parent and Idaho Refugee (dominant resistance) or Red Mexican (type of resistance unknown) was the female parent. Seed transmission to the F_1 progeny was determined as previously described.

The data (Table 3) show that a portion of seed of some resistant varieties transmitted virus when fertilized with pollen from infected plants. Less transmission occurred when infected susceptible females received pollen from the same resistant varieties. In contrast, no seed transmission occurred when Idaho Refugee or Red Mexican 34 served as the female parent and received pollen from infected susceptible plants. The type of resistance in Red Mexican 34 is not identified in the literature, but its reaction is like that of Idaho Refugee, which has the dominant type of resistance (1). Similarly, the type of resistance of the other varieties has not been identified, but it is likely that they have the recessive type derived from the Great Northern variety. If these assumptions are correct, the simplest explanation for the lack of seed transmission to F_1 progeny of Idaho Refugee and Red Mexican 34 is that nonsusceptibility of F_1 progeny, due to dominance of resistance, precludes seed transmission. On the other hand, F_1 progeny of varieties possessing the recessive type of resistance would be susceptible and therefore capable of receiving the virus in embryos from which they develop.

3) *Virus infection of floral parts.*—To determine which floral parts of infected bean plants are virus-infected, about 100 flowers were dissected into petals, pistils, and anthers; and composite samples of each portion were ground separately in 0.05*M* phosphate buffer, pH 7. Young bean plants were inoculated with each preparation. BV1 and its NY15 strain were recovered from all floral parts tested, whereas BV2 was recovered from pistils and petals but not from anthers. Inoculations made from infected leaf tissue were positive for all 3 viruses.

4) *Virus infection of embryos.*—Embryos (including cotyledons) from 50 mature seeds from plants infected with BV1, NY15, or BV2 were dissected out of the seed coats, ground separately in pH 7 phosphate buffer (1:10 w/v), and young bean plants were inoculated with the resulting preparations. Both BV1 and NY15 were present in embryos in infective amounts; but BV2 was not present in embryos from infected plants, or else the concentration was too low to be detected by mechanical inoculation of susceptible plants. Control inoculations with an extract from infected leaves resulted in infections with all 3 viruses.

5) *Germination of pollen from virus-infected plants.*—Crowley (7) theorized that infection of BV1 might interfere with the normal function of microspores and megaspores, but he found no significant difference in fertility of BV1- and BV2-infected plants and concluded that difference in seed transmissibility of the 2 was not due to abortion of microspores or

TABLE 4. Effect of virus infection on percentage of pollen germination and length of the germ tubes

Pollen source	Average germ tube length in μ	% of germination
BV2	94.6	52.3
BV1	116.3	68.8
Healthy	147.3	71.3
LSD 0.05	29.6	28.8

TABLE 5. Effect of temperature on BV1 transmission through the seed

Treatments[a]	Total F_1 plants	Infected F_1 plants	% of transmission
Plants grown continuously at high temperature (80–90°F)	123	70	56.7
Plants grown at low temperature (60–66°F), then at high temperature (80–90°F)[a]	117	72	61.5
Plants grown at high temperature (80–90°F), then at low temperature (60–66°F)[a]	78	43	55.3
Plants grown continuously at low temperature (60–66°F)	58	39	67.4

[a] Changes of temperature were made just before flowering period.

megaspores in BV2-infected plants. A more direct confirmation of Crowley's results was obtained herein by a study of the effect of virus infection on germination and growth of pollen grains.

The medium for germination tests of pollen from healthy bean plants and plants infected with BV1 or BV2 was 1.0% aqueous sucrose solution solidified with 1.5% agar. A drop of hot agar was placed on a clean glass slide coated with a thin layer of egg albumin. Pollen was dusted on the cooled agar, and the slides were kept in a moist chamber for 2 hours and then exposed for about 15 minutes to fumes of Flemming's fluid. Table 4 shows average pollen tube length and percentage of pollen germination.

Virus infection did not affect percentage of pollen germination significantly, but pollen tubes of pollen grains from BV1- or BV2-infected plants were significantly shorter than those from healthy plants (Table 4). The decrease probably reflected a debilitating effect on the whole plant rather than a direct effect of virus on pollen grains. This hypothesis is supported by our failure to demonstrate virus in anthers of BV2-infected plants.

6) *Test of seed extracts for virus inhibitors.*—Cheo (5) reported that, as seed approached maturity, the titer of southern bean mosaic virus in embryo tissue decreased and seed transmission fell from 80 to 2%. He postulated that the decrease was due to virus inhibitors that formed as seed matured. Tests were therefore conducted for inhibitors to BV1, BV2, or NY15 in mature bean seeds. Bean seeds from healthy plants were dissected to separate embryos from seed coats, and extracts from each were prepared. The tissue was macerated and mixed with distilled water—1:2 (w/v) for embryos, and 1:10 (w/v) for seed coats. The mixtures, after standing at room temperature for about 8 hours were centrifuged 10 minutes at 10,000 rpm (rotor 30-Spinco Model L). The supernatant fluids were mixed 1:1 (v/v) with juice expressed from infected bean plants, and 5 plants each of Small White and Sutter Pink were inoculated immediately.

All inoculated plants became infected, whether or not the inoculum contained the extracts, indicating that inhibitors of BV1 or BV2 were not present. It seems unlikely, therefore, that the difference in seed transmissibility of the 2 viruses is due to inhibitors in the mature seed.

7) *Effect of temperature on seed transmission.*—Fajardo (10) reported that a low percentage of seed transmission resulted if infection occurred after the blooming period and that plants infected during early vegetative growth produced more infected seed than plants infected at later stages. Therefore, any effect of environment on percentage of seed transmission probably would be expressed only if applied prior to the flowering stage. Crowley (7) studied the effect of temperature on percentage of seed transmission of BV1 and reported that no seed transmission occurred when the temperature was maintained below 68°F before the flowering period.

In the present study, BV1-infected plants were subjected to various temperature treatments following procedures similar to Crowley's (7). These treatments were: 1) plants grown continuously at 90°F; 2) plants grown at 90°F until bloom time and then transferred to 60-66°F; 3) plants grown continuously at 60-66°F; and 4) plants grown at 60-66°F until bloom time and then transferred to 80-90°F. In all, 32 pots were planted. The plants (Sutter Pink) were inoculated with BV1 at the 2-leaf stage. About a week later, when the plants were showing symptoms, 8 pots were placed in each of the environments described. Plantings of the harvested seed indicated that, under the conditions of this experiment, the percentage of seed transmission was not affected appreciably by the different treatments (Table 5).

The conflict with Crowley's results (7) is most likely due to varietal differences, since Sutter Pink appears to have a propensity for a high rate of seed transmission. Other factors may be difference in strain of virus used; unknown differences in environment; or transfer from one temperature to another. The last is unlikely, however, since results should then have agreed with Crowley's for plants grown at a low temperature throughout their vegetative period. Since the usual percentage of seed transmission of BV1 is about 50% (4, 14, 17), Crowley's results—his highest transmission was 25.4%—indicate that his environmental conditions or the bean variety used or both were suboptimal for seed transmission.

Discussion.—Numerous experiments have indicated that BV2 is rarely, if ever, seed-transmitted, although the seed-borne nature of BV1 and the related strain NY15 is well established.

BV1 and NY15 were similar in several aspects. Both were recovered from anthers, and both were present in the embryos of seed from diseased plants.

The following theories have been advanced (2) in an attempt to explain the lack of transmission of most viruses through the seed: 1) lack of vascular connections between mother plant and embryo; 2) inhibition by storage protein and other inhibitors in mature seed or embryo; 3) inability to infect and to survive in young embryos; and 4) inability to infect and survive in gametophytic tissues. Bennett (2) suggested that the lack of vascular connections might explain the lack of embryo infection by viruses restricted to vascular tissue, but it would not explain lack of embryo infection by viruses that invade parenchymatous tissue.

The hypothesis of virus inhibitors in the seed has not been fully substantiated by experimental evidence. Duggar (9) theorized that some viruses may be inactivated in the embryo by storage proteins. In support of this theory, he found that extracts from ground seed of tobacco and several other plant families partially inactivated tobacco mosaic virus. The concentration used, however, never completely inactivated the virus. Cheo's similar finding (5), with respect to southern bean mosaic virus was based on a decrease in seed transmission from about 80% in immature

embryos to about 2% in mature seeds, thus showing that inhibitory effect was not complete. In experiments here reported no inhibitors to BV2 were demonstrated in mature seeds of bean. It seems unlikely, therefore, that the lack of seed transmission of BV2 in bean is due to inhibitors.

One theory suggests that some viruses are not seed-transmitted because the embryo does not become infected. This hypothesis appears to hold true for BV2 while, on the other hand, the seed-transmitted BV1 and NY15 both were recovered from embryos. Bennett and Esau (3) demonstrated that curly top virus, which is not seed-borne, was present in the perisperm and seed coats of beet seed, increasing as seed matured. The virus, however, was not present in embryos. Present results seem to indicate that in most cases of seed transmission a virus must infect and survive in embryos.

Another of Bennett's theories suggests that non-seed-borne viruses, unlike seed-borne viruses, may be unable to infect the haploid tissues. Our data tend to support this theory, since pollen appeared to be virus-free when the plant was infected with BV2, but not when infected with BV1 and NY15. The latter 2 viruses are apparently also transmitted by ovules. Pollen transmission, reported by Reddick and Stewart (18), was confirmed by Nelson and Down (15), who postulated that the highest seed transmission possible was 50% (25% by pollen grains and 25% by ovules). The present results showed infections greater than 25% for either pollen or ovules in crosses in which only 1 parent was infected. Further, it is doubtful whether generalizations can be made about percentage of seed transmission of BV1 or NY15 since it is greatly affected by varietal differences (Table 2) and plant age at inoculation (10).

The growth of pollen tubes from both BV1- or BV2-infected plants was significantly slower than the healthy controls. Thus, effects on pollen-tube growth seem an unlikely explanation of the difference between the 2 viruses in seed transmissibility. The outstanding difference between these viruses seems to be the lack of virus in pollen or embryos from BV2-infected plants and its presence in pollen and embryos from plants infected with BV1 or NY15. The viruses probably differ also with respect to their presence in megaspores of infected plants but conclusive evidence on this was not obtained.

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THE ASSOCIATION OF CHLOROTIC LEAFSPOT VIRUS OF APPLE WITH DECLINE OF SPY 227 ROOTSTOCKS

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SUMMARY

Of 20 commercial apple varieties propagated on yearling budlings of the apple selection Spy 227 in 1958, 11 varieties developed normally, 1 variety made very weak growth, and 8 varieties either failed completely or were in advanced stages of decline by 1960. When indexed on Russian seedling R-12740-7A and *Malus platycarpa* (Long Ashton clone) in 1960, all 8 varieties that either failed or declined when propagated on Spy 227 were found infected with chlorotic leafspot virus (CLSV). Ten of 11 varieties that grew well on Spy 227 were free of CLSV, and inconclusive evidence was obtained that CLSV was not fully systemic in the remaining variety. Differences among various varieties in rate of decline when propagated on Spy 227 and the occurrence of a single variety free of CLSV, but growing weakly on Spy 227, probably indicates that more than 1 virus may be involved in Spy 227 decline. CLSV induces line pattern symptoms in *M. platycarpa;* of the 2 names currently proposed for this virus, CLSV holds priority over the synonym, *platycarpa* line pattern virus.

INTRODUCTION.—The clonal apple rootstock Spy 227 (USDA 227) initially appeared to possess several attributes of commercial value: ease of propagation, high vigor, and resistance to woolly apple aphids (7, 10). The uncongeniality of certain apple varieties with Spy 227 rootstocks, however, was observed by Tukey and Brase (7), who found that nursery trees of McIntosh and Delicious on Spy 227 rootstocks died within 3 years after progagation. In further experiments with 2 strains of McIntosh differing slightly in fruit type and rate of growth, these authors (8) found that 1 strain (McIntosh 227) was perfectly congenial with Spy 227 and developed into normal trees, but the second strain (McIntosh 1/35) failed completely when propagated on this rootstock.

Shaw and Southwick (6) listed the behavior of scions of 25 apple varieties or varietal strains propagated on Spy 227. Of these, 12 grew poorly or very poorly as 1-year budlings, while 13 made fair to excellent growth. Of the 13 varieties that grew well as 1-year budlings, only 3 grew vigorously the 2nd year; trees of the remaining 10 varieties either exhibited advanced decline or were dead by the end of the 2nd season after propagation. Yerkes and Aldrich (10) similarly listed the behavior of 114 apple varieties propagated on Spy 227 rootstocks: 20 varieties appeared to grow normally for 2 years but died in the 3rd season following propagation; 27 varieties grew normally for 1 season but died in the 2nd season; and 7 varieties grew poorly and died in the 1st season after propagation. The remaining 60 varieties, which included 5 selections of Delicious and 3 of McIntosh, developed into trees that remained normal in appearance over a 10-year period.

Gardner, *et al.* (1) demonstrated that vigorous 6-year-old orchard trees (var. Chenango and Golden Delicious) on Spy 227 rootstocks declined rapidly and died within 4 years after the insertion of buds from a source of Stayman Winesap that had previously proved lethal to Spy 227 in the nursery. In a parallel experiment, trees of Golden Delicious on seedling rootstocks similarly grafted from the same Stayman Winesap source remained apparently healthy. Gardner, *et al.* (1) suggested that their data could best be explained by the hypothesis that a toxic principle or virus lethal to Spy 227 was present in apple scions from certain sources. Weeks (9) transferred a factor lethal to Spy 227 rootstocks from an uncongenial clone (McIntosh R) to a previously congenial clone (McIntosh G) by bud inoculation, and also suggested that a virus was involved. Guengerich and Millikan (2) presented evidence that the virus lethal to Spy 227 might be the one which induced stem-pitting in Virginia Crab apple stocks.

The present paper will present evidence correlating the decline of Spy 227 with the presence of chlorotic leafspot virus (CLSV) in certain scion varieties.

EXPERIMENTATION.—In field experiments, commercial apple seedlings planted in the spring of 1957 were budded that August with buds of Spy 227. In August, 1958, 20 apple varieties or varietal strains were propagated on the resulting Spy 227 budlings. Five trees of each individual variety or varietal strain were propagated by inserting a single bud in each of 5 Spy 227 budlings about 10 in. above the point of union of Spy 227 with the seedling rootstock. In early 1959, Spy 227 growth above the varietal bud was removed, forcing the latter into growth. In this manner, composite trees (variety/Spy 227/seedling rootstock) were produced.

In greenhouse-indexing experiments, indicator trees of Russian seedling apple R-12740-7A (*Malus* sp.) (5), Spy 227, and *M. platycarpa* Rehd. (Long Ashton clone) were propagated on potted commercial apple

seedlings in February, 1960. A bud of the indicator variety was inserted about 6 in. above the ground line; and when it had united and commenced growth, 2 buds from a variety being indexed were inserted immediately below the indicator scion. The top of the commercial apple seedling was then removed, forcing the indicator scion into vigorous growth. All indicator trees were grown at alternating temperatures of 70-75°F (days) and 60-65°F (nights) until late June when close temperature control could no longer be maintained.

RESULTS.—*Spy 227 composite trees in the field.*—Data on budtake and growth of composite trees in 1959 and 1960 are presented in Table 1.

R-12740-7A in the greenhouse.—Individual varieties were indexed on triplicate indicator trees of R-12740-7A. In many cases, symptoms of chlorotic leafspot developed within 3-4 weeks after insertion of the index buds. Affected indicators abruptly ceased growth, and newly developing leaves failed to attain normal size and showed numerous pale-yellow circular areas from 1-4 mm in diameter. Leaves that developed yellow spotting were often unilaterally distorted (Fig. 1). Affected indicators remained stunted with essentially no further elongation of R-12740-7A terminals throughout the growing season. As a result, many of the index or seedling rootstock buds assumed dominance and grew vigorously; even when these were cut back severely, R-12740-7A terminals did not resume growth.

This syndrome is unquestionably that described by Mink and Shay (5) as chlorotic leafspot, and the causal virus will be termed CLSV.

M. platycarpa in the greenhouse.—Individual scion varieties were again indexed on triplicate indicator trees. The initial onset of symptoms was less clear-cut than in the case of R-12740-7A indicators, and

TABLE 1. Budtake and growth of apple varieties on Spy 227 interpieces, 1959-1960

Variety	Bud union	Bud growth	Growth 1959	Growth 1960
Cortland 2-17	5/5[a]	1/5[b]	weak	decline[c]
Rogers McIntosh 8-11	5/5	1/5	weak	decline
McIntosh 1/35	5/5	0/5	failed	decline[d]
Wellington Bloomless 4-32	5/5	3/5	weak	decline
Red Rome 5-10	5/5	2/5	weak	decline
Red 20-Ounce 8-10	5/5	0/5	failed	decline[d]
Red Wealthy 13-40	5/5	5/5	weak	weak
Vance Red Delicious 9-11	5/5	5/5	medium	medium
Wellington 8-26	5/5	5/5	medium	medium
Stark Earliest 20-36	5/5	5/5	medium	strong
Baldwin 2-3	5/5	5/5	strong	strong
Golden Delicious 2-39	5/5	5/5	strong	strong
Monroe 20-14	5/5	5/5	strong	strong
Lodi 6-37	5/5	5/5	strong	strong
McIntosh 227	5/5	5/5	strong	strong
Early McIntosh 11-32	5/5	5/5	strong	strong
Macoun 12-3	5/5	5/5	strong	strong
R. I. Greening 25-11	5/5	5/5	strong	strong

[a] Number of buds united/number inserted.
[b] Number of buds growing/number united.
[c] Symptoms of decline included poor growth, chlorosis, premature defoliation, and terminal dieback.
[d] Spy 227 terminals that grew showed poor growth, chlorosis, premature defoliation, and terminal dieback.

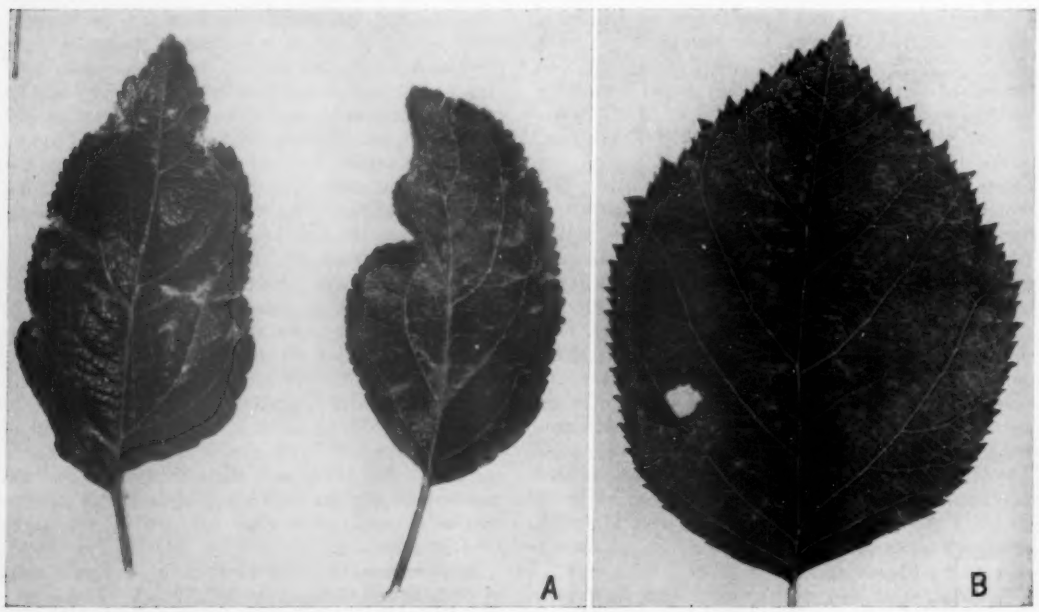

Fig. 1. Chlorotic leafspot symptoms in leaves. **(A)** Russian seedling R-12740-7A. **(B)** *Malus platycarpa*, Long Ashton clone.

many *M. platycarpa* indicators did not develop well-defined symptoms until 6-8 weeks after index buds were inserted.

Affected indicators were somewhat dwarfed with numerous, diffuse, chlorotic areas with poorly defined margins in the foliage. Occasionally, pale green oak-leaf patterns or rings subsequently developed in the younger leaves; but, in general, the prominence of foliage symptoms progressively decreased so that leaves produced in August were almost symptomless. Although there was no apparent tendency in the greenhouse toward premature abscission of leaves with

Fig. 2. Stunting and rosetting of R-12740-7A caused by CLSV infection. **(A)** Non-inoculated. **(B)** Inoculated with EM VII. **(C)** Inoculated with McIntosh 1/35. One of the McIntosh 1/35 index buds is growing vigorously (arrow).

TABLE 2. Indexing of scion varieties on Spy 227 indicators in the greenhouse, 1960

Variety indexed	Growth of Spy 227 indicators[a]	Symptoms
EM VII	6	RS[b]—defoliation
EM IX	9	RS —defoliation
Red 20-Ounce 8-10	9	RS —defoliation
Rogers McIntosh 8-11	23	RS
Golden Delicious 2-39	18	RS
Red Wealthy 13-40	5	defoliation
Vance Red Delicious 9-11	12	none
Early McIntosh 11-32	26	none
Macoun 12-3	22	none
Wellington 8-26	23	none
Lodi 6-37	22	none

[a] Mean of the terminal growth of 3 indicators (in.).
[b] RS = ring spotting.

symptoms, otherwise, the syndrome appears identical with that of "*platycarpa* line pattern" described by Luckwill and Campbell (3). None of our indicators developed the scaly bark syndrome described by these authors.

Spy 227 in the greenhouse.—Only 11 varieties were indexed in triplicate, but these included 2 varieties (EM VII and EM IX) that had not been propagated on Spy 227 in the field. Table 2 shows that several groups of Spy 227 indicators developed numerous, very small (1 mm or less in diameter), chlorotic rings in their foliage. Where such ring spotting was present, growth of Spy 227 indicator scions was usually poor, and many such indicators developed premature defoliation and incipient dieback of terminals.

Selected data (Table 3) show the relation of the indexing data obtained in the greenhouse and the growth data of Spy 227 composite trees in the field.

DISCUSSION.—The data presented in Table 3 show the correlation of chlorotic leafspot of R-12740-7A with line pattern in *M. platycarpa* and with decline of Spy 227. Except for a single variety (Golden Delicious 2-39), all varieties infected with CLSV either failed completely or declined when propagated on Spy 227 interpieces.

Gardner, *et al.* (1) suggested almost 15 years ago that decline of Spy 227 with certain apple varieties could best be explained by the postulate that a "toxic principle (virus)" was present in those varieties. These authors also pointed out some of the characteristics of the virus by stating: "If it is true that a virus is responsible for the puzzling stock and scion relationships outlined here, the virus simply exists as a masked entity and does no apparent harm to the varieties infected or to any known rootstocks except Spy 227 and certain . . . clones which have been developed from its seedlings."

CLSV conforms closely to this characterization. CLSV is completely masked in all infected commercial varieties examined and is similarly masked or latent in infected plants of the EM and MM rootstock series. Many of the scion varieties found infected have been propagated for more than 20 years on commercial apple seedlings and on the EM clonal rootstocks, without any indication that a virus was present. Similarly, several new apple varieties, initially free of CLSV, have been propagated on the rootstocks EM VII and EM IX without the development of symptoms, although all of the stocks of EM VII and EM IX tested have been found infected with CLSV. Luckwill and Campbell (3) point out that *platycarpa* line pattern virus (apparently synonymous with CLSV) is widely distributed in English apple varieties where it is completely latent and apparently harmless.

It is premature to conclude, however, that CLSV is completely innocuous. At least 3 apple clones in whose parentage R-12740-7A occurs have shown leaf mottle and pronounced stunting when propagated on EM VII or EM IX in our experiments, and Mink and Shay (5) have reported that 4 other clones derived from R-12740-7A developed chlorotic leafspot when inoculated. Thus, while CLSV is apparently innocuous to present commercial apple varieties, it could become of considerable economic importance if scab-immune varieties, derived from R-12740-7A, are introduced.

Two anomalies in the presented data require explanation: the normal growth of Golden Delicious 2-39 on Spy 227, even though CLSV was found in this variety in the greenhouse indexing; and the weak growth of Red Wealthy 13-40 on Spy 227, even though this variety was free of CLSV.

Although all 3 of the R-12740-7A indicators budded with Golden Delicious 2-39 developed severe symptoms of chlorotic leafspot, only 1 of 3 *M. platycarpa* indicators, similarly budded at the same time, developed symptoms of line pattern. These data suggest that CLSV was not fully systemic in the Golden Delicious tree

TABLE 3. A comparison of the reactions of 3 indicators to certain apple varieties

Variety	Reaction on *M. platycarpa*	R-12740-7A	Spy 227
Cortland 2-17	LP[a]	...	decline
Rogers McIntosh 8-11	LP	CLS	decline
McIntosh 1/35	LP	CLS	decline
Wellington Bloomless 4-32	LP	...	decline
Red Rome 5-0	LP	CLS	decline
Red 20-Ounce 8-10	LP	CLS	decline
EM VII	LP	CLS	decline
EM IX	LP	CLS	decline
Red Wealthy 13-40	—	—	weak
Baldwin 2-3	—	...	normal
Golden Delicious 2-39	LP	CLS	normal
Stark Earliest 20-36	—	—	normal
Monroe 20-14	—	—	normal
Lodi 6-37	—	—	normal
McIntosh 227	—	—	normal
Vance Red Delicious 9-11	—	—	normal
Early McIntosh 11-32	—	—	normal
Macoun 12-3	—	—	normal
Wellington 8-26	—	—	normal
R. I. Greening 25-11	—	—	normal

[a] LP = line pattern; CLS = chlorotic leafspot; ... = not indexed; — = virus-free.

from which scions were taken. Alternatively, this tree may have been infected with CLSV shortly after scions were propagated on Spy 227 in 1958.

The weak growth of Red Wealthy 13-40 scions on Spy 227 and the data presented in Table 2 probably indicate that more than 1 virus is present in the experimental material. Red Wealthy 13-40 apparently carries this 2nd virus free of CLSV, while EM VII, EM IX, and Red 20-Ounce 8-10 probably carry it and CLSV. Some support for this hypothesis is afforded by the extensive indexing of English apple varieties by Luckwill and Campbell (3), who found that inoculations from certain varietal sources induced dwarfing as well as line pattern in *M. platycarpa*. Since dwarfing was "usually . . . associated with the occurrence of line pattern, but . . . not invariably so," they concluded that "a distinct virus or virus complex is responsible for this reduction in vigor" (3). Different rates of decline in various varieties propagated on Spy 227 have been remarked by Shaw and Southwick (6) and Yerkes and Aldrich (10), and are present in our experiments. Such differences might result from the occurrence of different strains of CLSV; but, more plausibly, might be attributed to infection with 2 or more viruses, 1 of which is CLSV.

Our data indicate that a single virus induces line pattern in *M. platycarpa* and chlorotic leafspot in R-12740-7A. Whether this virus is identical with stem-pitting virus has not been determined. Pending possible identification, the term "chlorotic leafspot virus" proposed by Mink and Shay (4) should be preferred to "*platycarpa* line pattern virus" proposed by Luckwill and Campbell (3) because of publication priority.

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RESPIRATORY CHANGES DURING DEVELOPMENT OF RUST DISEASES

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SUMMARY

A systematic study at daily intervals was made of respiratory rates and pathways in diseases caused by rust fungi. Two taxonomically distinct systems of compatible host-parasite combinations were employed; race 56 of *Puccinia graminis tritici* on Little Club wheat and race 3 of *Uromyces phaseoli typica* on Great Northern bean. They were studied under similar controlled environmental conditions to determine whether there are changes in respiratory metabolism which are common to rust infections. Special attention was paid to the status of host tissue and uniformity and intensity of infection.

Both bean and wheat leaves responded to infection by rust fungi with increases in respiratory rates and by a sharp drop in C_6/C_1 ratio during later stages of the disease syndrome. Neither the Pasteur effect nor sodium fluoride inhibition is sufficiently consistent to describe respiratory changes induced by infection. Since lowering of the C_6/C_1 ratio appears to be characteristic of rust infection, the techniques and calculations employed were examined in detail. The data indicate a necessity for corrections for differences in respiration rates between healthy and diseased tissue in determining C_6/C_1 ratios and that the methods of calculation of percentage recovery of isotope are important in interpreting results of recovery studies.

In contrast to some previous reports in the literature, the change in metabolism indicated by the decline in C_6/C_1 was not inversely proportional to respiratory rates. Respiratory rates and C_6/C_1 ratios were similar in both healthy and diseased plants during the first 3 or 4 days after inoculation, but

rates rose shortly before or at the time flecks were apparent. The drop in C_6/C_1 ratio occurred abruptly at least 24 hours later. In all instances but one, the drop in C_6/C_1 ratio was noted only after spores had been observed, suggesting a close relationship between sporulation and a change in metabolism. Decline in C_6/C_1 ratio was shown to be due primarily to a lowering of the recovery from the C_6 rather than the C_1 position, except in later stages of heavy infections.

These results were obtained in a number of separate experiments, despite fluctuations in host metabolism both diurnally and in successive daily samples. The significance of observed changes is discussed with respect to 1) development of rust fungi on susceptible hosts, 2) nature of the metabolic pathways, and 3) mechanisms inducing a change in C_6/C_1 ratios.

INTRODUCTION.—An increase in oxidative metabolism has been documented amply for a wide variety of hosts infected with rust fungi (1, 7, 8, 9, 15, 17). Less well documented, but found to occur with 2 distinct types of rust diseases, is an apparent change in the pathways of the increased respiration. In the case of safflower rust, 3 lines of evidence were presented which suggested that the oxidative pentose pathway (hexose monophosphate shunt) was of major importance during sporulation (8). The principal line of evidence was a lack of sensitivity of infected hypocotyls to sodium fluoride, suggesting a by-pass of the Embden-Meyerhof-Parnas route of catabolic carbohydrate metabolism. The lack of fluoride inhibition occurred at the time sporulation was initiated. The possible nature of the by-pass was indicated by a lowering of the relative recovery of isotopic carbon dioxide from the C_6 and C_1 positions of glucose (C_6/C_1 ratio). Since this ratio was lowered markedly in a single determination during the sporulation process, indicating a preferential decarboxylation of the C_1 aldehydic group of glucose, the pentose pathway previously (2) detected in plants was considered likely. These 2 lines of evidence were consistent, in mechanism and time of occurrence during parasite development, with the inhibition of the Pasteur effect previously pointed out by Allen (1) to be associated with obligate parasitism.

Shaw and Samborski (15, 17) published data on foliar infections of stem rust on wheat, suggesting a similar change in metabolism. However, the sodium fluoride effect was inconsistent in their experiments and raised a question as to the significance of the change in C_6/C_1 ratio in diseased tissue. Their data suggested an increased rate of decarboxylation of C_1 which may result in formation of 5-carbon derivatives, but which are channeled via 3-carbon units through steps of the Embden-Meyerhof-Parnas pathway which lead to the Krebs cycle. This type of reaction scheme represents a limited by-pass of the early stages of glycolysis, rather than the cyclic operation (8) of oxidative pentose breakdown suggested for safflower. The data presented for wheat suggested also that the change in metabolic pathway was associated with the rise in respiratory rate and might not be associated directly with the process of sporulation.

The preliminary evidence that there possibly are metabolic pathways characteristic of rust-affected plants offers an avenue of entrance into the complex problems of obligate parasitism, the nature of resistance, and utilization of chemicals for disease control. The existing data, however, are too meager to establish the general validity of such metabolic changes in infections by rust fungi. The metabolism of both host and host-parasite complex is dependent on environmental conditions and on inherent genetic and cellular constitution, 2 important considerations in view of the variability of infection types and the physiologic specialization in rust. In turn, the metabolism of a single host-parasite combination may be dependent on variables such as type and age of tissue invaded and infection intensity. In the analysis of metabolic changes associated with pathogenesis, establishment of a pattern in development of the change has not only biological significance, but, if a pattern exists, it must be considered in examining individual biochemical or physiological steps. Otherwise, inconsistent and contradictory evidence will be obtained.

The present study was initiated to determine if some previously suggested criteria (Pasteur effect, inhibitors, and C_6/C_1 ratio) were reliable enough for critical studies of the metabolic consequences of infection. A second aim was to ascertain the effect of certain environmental and biological variables in response to infection. Finally, an attempt was made under controlled environmental conditions to see if changes in metabolism could be correlated with specific stages of development in susceptible host-parasite combinations.

MATERIALS AND METHODS.—*Growth and inoculation of host plants.*—Bean plants (*Phaseolus vulgaris* var. Great Northern 59) were inoculated with race 3 of *Uromyces phaseoli typica* by dusting with a talc-uredospore mixture approximately 2 weeks after planting. Wheat plants (*Triticum vulgare* var. Little Club) were inoculated with race 56 of *Puccinia graminis tritici* by the same method. Dusted plants, but not control plants, were placed in a glass-covered dew chamber overnight at 20-22°C with weak fluorescent light for at least a portion of the incubation period. It would be desirable to place noninoculated plants under the same conditions during incubation, but attempts to do so resulted in appreciable infection owing to the presence of spores in the air. The differences in environment between control and inoculated plants were most pronounced in experiments with greenhouse-grown bean. In several experiments, incubated, nondusted plants were compared with plants kept on the greenhouse bench, but no significant differences in respiratory rate

were observed. Several experiments with bean and all experiments with wheat were carried out on plants grown under controlled conditions of 20-22°C and fluorescent lighting of approximately 1,200 ft-c for 12-14 hours. In these experiments, incubation conditions were equivalent, except for moisture and a reduction in light intensity due to condensation on the glass cover of the incubation chamber.

In all inoculations, special attention was paid to ensuring a dense and uniform infection on only the leaf tissue to be studied. Infections on other leaves might lead to problems due to translocation of substrates and increase the variability of results. In sampling the tissue, the entire leaf was excised and cut into segments (5 mm sq for bean leaves and 5 mm long for 1st leaf of wheat). The sampling process was standardized so that the time of excision and the length of time required for preparation of the samples were the same for each day. Such procedures will minimize diurnal fluctuations in metabolism. A high infection intensity was desired since alterations in metabolism may occur before signs and symptoms of disease appear and we have assumed that heavy infection would reveal such differences more readily. Since the entire leaf of infected plants was selected, uniformity of infection was required in order to ensure comparable tissues from day to day, especially before symptoms appeared.

The time required for development of any parasite is dependent on both environmental and internal host conditions; the data presented are expressed both in terms of days elapsed from inoculation and stage of parasite development. The symbol F denotes strong flecking while S refers to a trace of sporulation, macroscopically visible. After sporulation was observed, the approximate degree of sporulation was given in percentages. Such a system was not as accurate as desired since data were obtained only at the the time of collection of the sample. In many cases, particularly with rust of wheat, the time lapse between initial flecking and complete sporulation was 36-48 hours, a period normally only sampled twice.

Respiratory measurements.—Standard Warburg procedures, described previously (7) were employed. After rinsing a 1-3 gm sample of tissue, 200-500 mg (fresh weight) of tissue were placed in 1.5-2.0 ml 0.1 *M* KH_2PO_4 buffer in duplicate or triplicate vessels. The buffer contained 12.5 or 25 μM glucose/ml. When inhibitors, hormones, or anaerobic carbon dioxide production was studied, a 1-hour equilibration period was observed before rates were measured. Rates were measured at 21°C, the temperature maintained during growth, except in the case of bean leaves obtained from the greenhouse when a temperature of 26.6°C was considered most appropriate for respiratory measurements. The data are expressed as Q_{O_2}, Q_{CO_2} (μl per mg dry wt per hour in air), or $Q^{N}_{CO_2}$ (μl anaerobic CO_2 per mg dry wt per hour under nitrogen). In some instances where dry weight was not determined, the gas exchange is presented as μl per 100 mg fresh wt per hour.

C_6/C_1 ratios.—The rationale and limitations of the Bloom and Stetten procedure for evaluating the relative contributions of the oxidative pentose pathway (PP) and the Embden-Meyerhof-Parnas (EMP) pathway of glycolysis have been discussed previously (2, 4, 10). In our procedure, segments of normal and diseased tissue were shaken for 2-5 hours in 0.1 *M* KH_2PO_4 buffer containing 12.5 or 25 μM/ml of glucose labeled with C^{14} either in the C_1 or C_6 position. Usually 300 to 500 mg fresh weight of tissue were floated on 4-5 ml of solution. In any series of daily measurements, sufficient stock solutions of reagents were made so that all determinations were directly comparable. This eliminated possible errors due to changes in solution composition. In 2 experiments with bean leaves, the radioactive carbon dioxide produced by respiration was trapped in 0.1 ml of 10% KOH in the side arms of specially constructed Warburg vessels of large volume. In all other cases, the KOH was placed in a centrally-positioned Pyrex cup suspended from a rubber stopper in the neck of 50 ml Erlenmeyer flasks. At the end of a specified time, tissue was killed by injection of trichloroacetic acid with a hypodermic needle through a side arm capped with a serum bottle stopper. After shaking an additional 30 minutes to allow for diffusion of CO_2 to the center well, the radioactive KOH was transferred, with 3 rinses of distilled water, to stainless steel planchets. The planchets were fitted with a piece of circular lens paper, 0.1 ml of 10% glucose was added, the planchets were rapidly dried at 90°C, and left in the oven overnight. Planchets were counted while still warm for at least 6 minutes with an end window Geiger tube. Initial radioactivity of glucose-1-C^{14} or glucose-6-C^{14} was determined in the same way.

Although these procedures resulted in some exchange loss of isotope, the experiments are on a comparative basis and absolute recoveries are not necessary. Furthermore, exchange losses occur at a rapid rate even when barium carbonate assays for radioactivity are used. The method employed in this study is much more rapid and satisfactory for large numbers of samples. In our experience, if the planchets are dried rapidly (within 1-2 hours) and counted immediately after removal from the oven, the variation between replicates is not greater than 5%.

Two or usually 3 replicate flasks were used in determining the recovery of isotopic carbon dioxide from glucose-1-C^{14} or -6-C^{14}. In our experience, the use of Warburg flasks for such experiments is not satisfactory since agreement is poor. The differences in recovery are consistent and may reside in differences in geometry among flasks.

There are several methods for calculating C_6/C_1 ratios, of which 2 are summarized in Table 1. The method selected considerably influences the interpretation of data. In the first method, radioactivity in

TABLE 1. Comparison of methods for calculation of C_6/C_1 ratios

Method		H (4½ hr)[a]		D (3 hr)		C_6/C_1		Relative recovery D/H	
		C_1	C_6	C_1	C_6	H	D	C_1	C_6
1. Exogenous recovery	Total CPM	723	466	1411	444				
	% recovery of added glucose[b]	1.68	.91	3.28	.86	.54	.26	1.95	.95
	Total μM CO_2 produced	8.8		21.1					
2. Specific activity of CO_2	CPM/μM CO_2	82	53	67	21				
	CPM/μM CO_2 / CPM/μM C_1 or C_6	19.0	10.4	15.6	4.1	.55	.26	.82	.39

[a] 0.3 gm of healthy (H) and diseased (D) wheat leaves shaken for 4½ and 3 hours, respectively, in triplicate flasks of 4 ml 0.1 M KH_2PO_4 containing 100 μM glucose-1-C^{14} or glucose-6-C^{14}.
[b] Total activity in each flask: C_1-43,000 CPM, C_6-51,000 CPM.
[c] C_1 equals 430 CPM/μM; C_6 equals 510 CPM/μM.

counts per minute (CPM) for the carbon dioxide collected from glucose-1-C^{14} or glucose-6-C^{14} is divided by the radioactivity (CPM) of the added sugar initially present (Table 1). This quantity (percentage of recovery from exogenous total activity) for C_6 is divided into the percentage of recovery for C_1 to give a C_6/C_1 ratio of 0.54 for healthy tissue and 0.26 for diseased tissue. This is the method employed by Shaw and Samborski (17) in their studies of wheat rust and mildew, except they used equal dry wt samples and measured radioactivity after equal time intervals for both healthy and diseased tissue.

Simple calculations such as this, however, ignore the important fact that in many instances the diseased tissue is respiring at a much more rapid rate than healthy tissue. As has been pointed out (8), the longer an experiment of this type is allowed to run, the more complete will be the utilization of endogenous substrate and consequently the C_6/C_1 ratio will approach unity as a function of time. Obviously, a high rate of respiration is equivalent to increasing the time of exposure to substrate, since the degree of cycling of substrate is greater than in a tissue of low respiration rate.

This is shown quite clearly in Fig. 1. Rusted and healthy bean leaves were killed at 2, 4, and 6 hours after initial exposure to radioactive glucose. The influence of time on the C_6/C_1 ratio is most marked with the diseased plants which were respiring at a rate 70% greater than the controls. With time, the difference (D) in ratios between the 2 tissues became less marked.

In an ideal assessment of respiratory pathways, it would be most suitable to collect radioactive carbon dioxide after both healthy and diseased tissue had respired equal amounts of carbon dioxide. Except where noted, healthy tissue was allowed to respire for 4 hours before killing and diseased tissue was killed at an earlier time which was calculated to give a comparable amount of carbon dioxide. No tissue was killed, however, before 2 hours, since this might result in complications due to diffusion and uptake of sugar by the tissue.

A second aspect of these determinations involves the meaning of the term "percentage recovery of isotope." Some significance (18) has been attached to the values reported for diseased tissue (1). Table 1 shows that the method of calculation employed has a pronounced bearing on the interpretation of data obtained in a single experiment.

In the experiment (Table 1), the healthy tissue produced 8.8 μM of carbon dioxide in 4½ hours, while diseased tissue produced 21.1 μM in only 3 hours. The total CPM recovered (1st line of Table 1) was recalculated as CPM/μM CO_2 and then divided by the initial CPM/μM of glucose with radioactive label at either C_1 or C_6.

The percentage of recoveries determined this way presents a different picture when healthy and diseased tissues are compared, even though the C_6/C_1 *ratios*

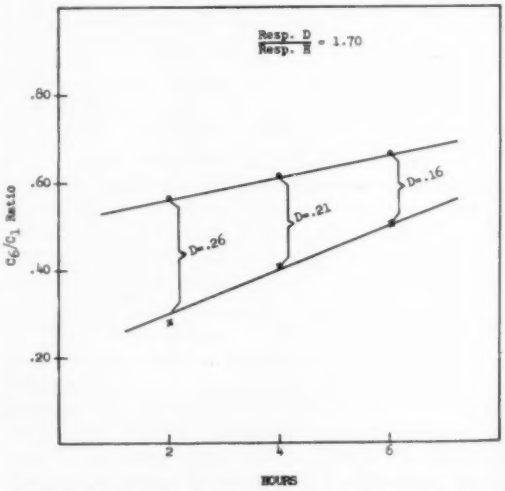

Fig. 1. Changes in C_6/C_1 ratios as a function of time of exposure to radioactive glucose; 0.3 g of healthy (upper curve) and diseased (lower curve) in 25 μM specifically labeled glucose. Diseased plants respiring at a rate of 70% higher than the healthy plants. See text for details.

TABLE 2. Respiratory changes in rust-affected 1st leaf of wheat[a] (Infection 159 p/leaf)

Days after inoc.	Stage	Gas exchange						Isotope recovery					
		Q_{O_2}		Q_{CO_2}		$Q_{CO_2}^{N_2}$		C_1		C_6		C_6/C_1	
		H	D	H	D	H	D	H	D	H	D	H	D
3		2.1	2.0	3.0	2.7	.83	.81	10.5	8.5	5.0	3.7	.48	.44
4		2.2	2.5	3.1	3.6	1.16	1.03	7.5	7.3	3.3	3.1	.44	.42
5	F[b]	2.5	2.9	3.2	3.6	1.04	.93	8.7	5.3	4.1	2.3	.47	.44
6	F(S)	2.1	3.0	2.9	3.8	.74	.89	7.0	6.3	2.8	2.3	.40	.37
7	40% S	2.1	4.0	2.8	4.8	.53	.62	6.6	5.1	2.0	1.1	.30	.22
8	80% S	1.8	4.1	2.4	5.3	.58	1.01	4.0	3.3	1.5	.6	.38	.20
10	100% S	1.9	5.0	2.7	5.6	.71	1.02	5.4	6.6	1.5	.7	.28	.11
11		1.7	3.5	2.3	4.3	.89	.78	12.2	13.1	2.3	1.2	.19	.09

[a] Grown at 21°C, 12-hour daylength; collected 1 hour after the start of light period.
[b] "F" denotes heavy flecking, "S" sporulation of pustules in approximate percentage, (S) sporulation in trace amounts. "H" denotes healthy, "D" denotes diseased.

are the same by both methods. In the exogenous recovery method of calculation, it appears that the difference in C_6/C_1 is due to increased recovery of C_1 carbon dioxide in the diseased tissue, while in the second method it would appear that a reduction in C_6 recovery is the reason for the decline in ratio (Table 1, last 2 columns). It should be emphasized that the discrepancies between these 2 methods would be more pronounced if 1) healthy tissue had been killed at the same time as diseased tissue and 2) a dry wt rather than wet wt basis had been employed.

Throughout the paper, the 2nd method (specific activity of carbon dioxide) is employed unless noted otherwise.

RESULTS.—*Respiration rates.*—Heavy infection of race 56 on the 1st leaf of Little Club wheat did not materially change respiratory rates until the 3rd or 4th day after inoculation (Tables 2, 3). In both cases, the increase in respiration occurred before flecking. Maximum rates were obtained when sporulation was well advanced. A greater percentage increase was found in the heavier infection (Table 2), but this may have been partially the result of a lower level of metabolism in the control plants, which were several days older when inoculated than the plants of Table 3.

Trifoliate (Table 4) and primary (Table 5) leaves of greenhouse-grown bean also showed a response in respiration at the time when signs of the pathogen were first apparent. It is to be noted, however, that with trifoliate leaves there was an initial stimulation which persisted until flecking, while with primary leaves the stimulation had disappeared by the 4th day. The reason for initial stimulation is not known, but it may have been associated with conditions during inoculation in conjunction with the process of infection. As mentioned previously, noninoculated plants placed under the same conditions did not differ significantly from controls left on the greenhouse bench. In addition, it is difficult to interpret such differences as arising and persisting for several days solely from a brief alteration in environment, especially in view of the marked fluctuations in temperature and light which occur naturally in the greenhouse.

A comparison of 2 levels of infection on very young primary leaves was obtained under the same constant environmental conditions as wheat (Fig. 2). Under these conditions, development of rust was extended so that the fleck stage was evident for 3 days without macroscopic evidence of sporulation and 3 additional days were required before sporulation was completed. Although the difference in infection intensities was approximately 2-fold, both infections were extremely heavy and could be distinguished only by actually counting the pustules. Respiratory curves were similar for both infections, but the heavier infection resulted in larger increases during the initial rise of respira-

TABLE 3. Respiratory changes in rust-affected 1st leaf of wheat[a] (infection 114 p/leaf)

Days after inoc.	Stage	Gas exchange						Isotope recovery					
		Q_{O_2}		Q_{CO_2}		$Q_{CO_2}^{N_2}$		C_1		C_6		C_6/C_1	
		H	D	H	D	H	D	H	D	H	D	H	D
1		2.6	2.7	3.1	3.1	1.00	.93	8.5	8.0	3.3	3.4	.39	.42
2		2.4	2.5	2.7	2.7	.64	.61	7.6	8.9	3.3	3.7	.43	.42
3		2.6	2.9	3.6	3.3	.62	.59	9.7	6.9	4.0	2.4	.41	.35
4		3.0	3.6	3.1	3.8	.52	.68	3.4	3.8	1.3	1.6	.38	.42
5	F	2.4	3.1	2.7	3.5	.62	.71	6.0	4.6	2.5	1.7[c]	.42	.37
6	F (S)	2.4	3.3	2.8	3.7	.97	.99	5.2	4.1	2.3	1.5	.44	.37
8	70% S	2.2	4.0	2.7	4.7	1.00	1.19	4.2	6.6	1.6	1.2	.38	.18

[a] Grown at 22°C, 14-hour daylength, collected 3 hours after start of light period.
[b] "F" denotes heavy flecking, "S" sporulation of pustules in approximate percentage, (S) sporulation in trace amounts. "H" denotes healthy, "D" denotes diseased.
[c] Only 1 replicate of C_6.

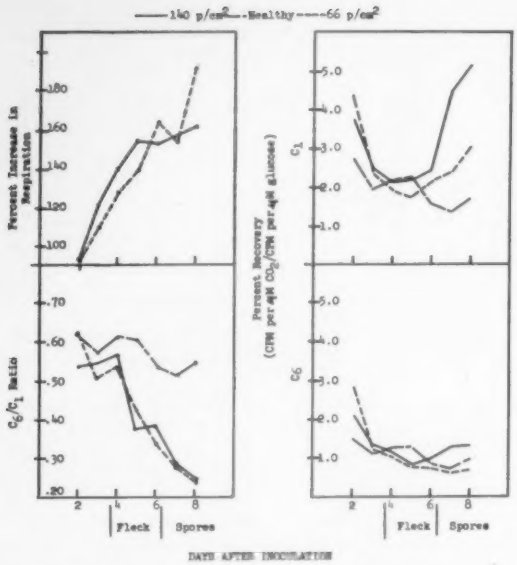

Fig. 2. Percentage of increase in respiration (upper left), C_6/C_1 ratio (lower left) and percentage of recoveries (right) for 2 levels of infection of bean rust. See text for details.

tion. In later stages, lighter infection appeared to induce a slightly greater response.

Respiratory quotients.—As respiration rates of infected wheat and bean tissue increased (Tables 2-5), the R.Q. fell below that of the controls. This effect can be most readily explained by the fact that absolute values of increased oxygen consumption paralleled absolute values of carbon dioxide production in diseased tissue; that is, the R.Q. of the increased respiration was approximately 1 (7). Since the R.Q. of healthy tissue was usually above 1, the diseased R.Q. will be numerically between 1 and the value for healthy tissue. Lowering of the R.Q. of diseased tissue was most pronounced during flecking and early sporulation.

An analysis of the significance of changes in R.Q. is complicated by fluctuations both diurnally and in successive daily samples of uninfected bean and wheat hosts. The complications are illustrated in Table 6. Tissue was sampled 10 minutes after the start of a 14-hour photoperiod and 5 hours later. The R.Q. of the morning healthy tissue was constant during the 1st 3 determinations but was appreciably higher on the 6th day after inoculation. On this day, there was also a sharp drop in oxygen consumption from the previous 3 days. The magnitude of both the decline in oxygen consumption and the increase in R.Q. was well above any possible error and the reality of the change is supported by consistently low rates for subsequent morning samples.

Additional evidence for abrupt changes in respiration is obtained when data for afternoon samples of healthy tissues are compared. The R.Q. was always higher in the afternoon and the change was due to a pronounced increase in carbon dioxide production without a marked change in oxygen consumption. On the afternoon preceding the lowering of oxygen consumption, the R.Q. of healthy tissue showed a diurnal fluctuation of much greater magnitude than the previous 2 days and the R.Q. was higher in the final afternoon sampling.

Diseased tissue also showed a marked diurnal fluctuation in R.Q., but it is to be noted that the magnitude of the changes before signs of infection is greater than for healthy tissue. On the 3rd and 4th days after inoculation, morning samples of diseased tissue were only slightly higher in R.Q. while afternoon samples were measurably higher. On the 5th day, when flecks were clearly evident, the R.Q. was below the healthy controls.

TABLE 4. Respiratory changes in rust-affected 1st trifoliate leaves of bean (GN59)[a] (Infection 33 p/cm²)

Days after inoc.	Stage	Q_{O_2}		Q_{CO_2}		$\frac{N_2}{Q_{CO_2}}$		% recovery C_1		% recovery C_6		% recovery C_6/C_1	
		H	D	H	D	H	D	H	D	H	D	H	D
1		2.7	3.3	3.1	3.7	.30	.61						
2		2.8	3.2	3.1	3.7	.28	.48						
3		2.5	2.9	2.8	3.2	.39	.51	.27	.30	.12	.19	.44	.63
4		2.4	2.7	2.5	3.0	.33	.44	.27	.26	.14	.16	.52	.61
5		2.5	2.9	2.8	3.0	.48	.48	.20	.19[c]	.081	.10	.41	.53
6	F[b]	2.3	2.7	2.7	3.0	.46	.48						
7	F	2.4	3.3	2.7	3.5	.41	.44	.22	.26	.10	.14	.45	.54
8	F (S)	2.1	3.1	2.4	3.3	.29	.40	.29	.30	.11	.11	.38	.37
9	50% S	2.2	3.2	2.3	3.3	.38	.48	.31	.26	.14	.095	.45	.36
10	100% S	2.2	3.2	2.3	3.4	.24	.34	.36	.26	.16	.085	.44	.33
12		1.9	3.4	2.2	3.8	.36	.45	.32	.22	.14	.076	.44	.35
14		1.6	3.0	1.7	3.2	.14	.35						
17		1.8	3.3	1.9	3.4	.15	.45						

[a] Collected at 2:30 PM from greenhouse plants in March. Temperature range 70-85°F.

[b] "F" denotes heavy flecking, "S" sporulation of pustules in approximate percentage, (S) sporulation in trace amounts. "H" denotes healthy, "D" denotes diseased.

[c] 1 replicate.

TABLE 5. Respiratory changes in rust-affected primary leaves of bean (GN59)[a] (Infection 65 p/cm²)

Days after inoc.	Stage	Q_{O_2}		Q_{CO_2}		$Q^{N_2}_{CO_2}$		% recovery C_1		% recovery C_6		% recovery C_6/C_1	
		H	D	H	D	H	D	H	D	H	D	H	D
1		3.0	4.0	4.0	4.9	.56	.56						
2		2.9	3.3	3.6	4 1	.40	.40	.19	.23	.072[d]	.10	.38	.44
3		2.9	3.4	3.8	4.4	.38	.40	.25	.29	.10	.11	.40	.38
4		3.0	3.0	3.6	3.5	.47	.28	.20	.21	.094	.089	.47	.42
5		2.4	2.4	2.9	2.9	.29	.24						
7	F[b](S)[c]	2.1	2.8	3.1	3.0	.50	.34	.30	.27	.14	.11	.47	.41
8	80% S	2.2	2.8	2.4	3.1	.25	.32	.35	.28	.12	.067	.34	.24
9	100% S	2.4	3.8	2.7	4.3	.29	.58						
10		2.5	3.0	2.7	3.2	.27	.26	.34	.59	.16	.17	.47	.27
12		2.4	3.7	2.6	3.9	.22	.42	.35	.42	.15	.13	.43	.31
14		2.4	3.5	2.5	3.7	.23	.51						

[a] Collected at 12:30 PM from greenhouse grown plants in April. Temperature range 68-95°.
[b] "F" denotes heavy flecking, "S" sporulation of pustules in approximate percentage, (S) sporulation in trace amounts. "H" denotes healthy, "D" denotes diseased.
[c] Plants flecked on day 6.
[d] One replicate.

Similar effects for healthy plants and during disease development can be observed in data of previous tables on respiration.

Anaerobic carbon dioxide production and the Pasteur effect.—In earlier studies of rust of safflower (7) and wheat (15, 17), it was shown that healthy tissue normally exhibited a Pasteur effect (P.E.) but rust-affected tissue did not. In both these instances, the magnitude of P.E. was measured by calculation of the ratio of anaerobic to aerobic carbon dioxide production ($Q^{N}_{CO_2}/Q_{CO_2}$). Healthy wheat and safflower had ratios greater than 0.33, indicating a P.E., while rusted tissue had values below 0.33. In the present study, normal tissue of bean and wheat did not consistently show a marked P.E.; calculation of $Q^{N}_{CO_2}/Q_{CO_2}$ (Tables 2-5) on many days will give a ratio below 0.33 for healthy tissue. Most usually the ratios lie between 0.2 and 0.3.

There are 2 possible sources for the discrepancies between the present data and those of Shaw and Samborski (17). First, data in the tables were obtained in the 2nd hour of manometer readings following exposure of tissue to nitrogen. When oxidatively metabolizing tissue is transferred to anaerobic conditions, there frequently is a burst of carbon dioxide production which subsequently declines, reaching a steady-state rate toward the end of the 1st hour. This is shown quite clearly for wheat in the data of Samborski and Shaw (15). These authors calculated their P.E. from data obtained during the 1st hour, while we have chosen to emphasize the steady-state value during the 2nd hour. A 2nd possible explanation lies in the metabolic activity of the tissue itself. The R.Q.'s of healthy tissue in the present study ranged from 1.10 to as high as 1.47, but the highest R.Q. shown by Shaw and Samborski is 1.03, although the values for Q_{O_2} were approximately the same. The high R.Q.'s for the data of the tables would indicate high levels of organic acids. This subsequently was established for tissues grown under these conditions. Irrespective of cause, the relatively large excess of carbon dioxide production per unit of oxygen consumption in healthy tissue would tend to minimize a P.E. when measured by the method used in this paper.

In diseased wheat, there is a general tendency for the ratio $Q^{N}_{CO_2}/Q_{CO_2}$ to be lower than healthy tissue during the period of maximum respiratory activity, even though the diseased tissue has a higher absolute rate of anaerobic carbon dioxide production. With

TABLE 6. Diurnal fluctuations in CO_2 production and C_6/C_1 ratios in healthy and rust-affected wheat

Days after inoc.	Stage[a] AM[b]	Stage[a] PM[c]	$\mu l\ O_2$/100 mg tissue H AM	H PM	D AM	D PM	R Q H AM	H PM	D AM	D PM	C_6/C_1 H AM	H PM	D AM	D PM
3			15	16	14	16	1.16	1.31	1.18	1.44	.43	.39	.41	.39
4		LF	15	16	14	15	1.14	1.24	1.17	1.33	.42	.36	.40	.35
5	MF	HF	15	15	19	18	1.13	1.47	.92	1.33	.48	.31	.46	.37
6	10%S	65%S	10	12	22	26	1.30	1.44	1.12	1.27	.38	.39	.28	.31
7	95%S		12		27		1.17		1.19		.43		.22	
9	100%S		13		34		1.23		1.12		.41		.18	

[a] "L," "M," "H," are light, medium, and heavy degrees of flecking; "F" indicates flecks; 'S" indicates visible spore production. Final infection: 66.8 pustules/leaf.
[b] AM samples collected 10 minutes after light period initiated.
[c] PM samples collected 5 hours after light period initiated.

greenhouse-grown bean, this was not the case. The $Q^{N}_{CO_2}/Q_{CO_2}$ was substantially the same for both healthy and diseased tissue indicating, in contrast to wheat, that the disease-induced anaerobic carbon dioxide production was proportional to the increase in aerobic carbon dioxide when compared to healthy tissue.

C_6/C_1 ratios.—The percentage of recoveries for carbon dioxide released from the 1st and 6th carbons of glucose, shown in Tables 2 and 3 for wheat and Tables 4 and 5 for bean, were calculated from the specific activity of carbon dioxide. Examination of the data for healthy tissue shows that there frequently were marked fluctuations in recovery from day to day. Although minor fluctuation (5%) conceivably could be attributed to errors in the biological and isotopic techniques, there are abrupt changes (Table 2, 7, 8 days; Tables 3-4, 5 days; and Fig. 2, 5, 6 days) which can not be readily ascribed to experimental procedures. In Fig. 2, for example, the drop from the 5th to 6th days represents nearly a 40% difference in recovery; in absolute values, a drop of about 400 CPM for C_1 and 300 CPM for C_6. Bean tissue either from the greenhouse or grown under the same conditions as wheat showed much less utilization of exogenous glucose.

Similarly, the C_6/C_1 ratios of healthy tissues of wheat and bean showed fluctuations from day to day which were not correlated with any known experimental fault. Changes in C_6/C_1 ratios were not correlated consistently with marked changes in the percentage recovery of isotope. Furthermore, changes in ratio were not associated with changes in either C_1 or C_6 recovery: in some instances, a change in ratio was due to a change only in C_1 or in C_6 recoveries; in others, both C_1 and C_6 recoveries were altered to give a ratio different from the previous day. Many of the changes were slight (for example, days 3, 4, and 5, Table 2) and could readily have occurred through compounded experimental error of consecutive runs. The abrupt changes on subsequent days in this Table are more difficult to interpret.

When allowance is made for different rates of carbon dioxide production at the start of the light period and 5 hours later, there appears to be a slight difference in ratio in healthy tissue, the early collection being consistently higher (Table 6). These data also clearly show a transition in ratios in morning and afternoon samples for the 5th day and the morning sample of healthy tissue on the 6th day after inoculation, at the time when alterations in R.Q. also were pronounced.

Despite the characteristic fluctuation of noninfected tissue, the C_6/C_1 ratios for diseased tissue are consistent in that they are markedly reduced once signs and symptoms of the pathogen are observed. It is to be noted, however, that the drop in ratio is not synchronous with the rise in respiratory rate of diseased tissue. With all combinations of host tissue and levels of infection, the increase in respiration rate precedes a shift in C_6/C_1 ratio. Further, the initial drop in ratio as a consequence of infection is quite sudden, occurring within 24 hours, while the increase in respiratory rate occurs over a span of several days.

In our experience with infection on the 1st leaf of Little Club wheat, including many experiments not shown here, significant differences in C_6/C_1 ratio between healthy and diseased tissue have been found only when visible sporulation has been observed at the time samples were collected. Invariably, respiratory rates have been higher for at least 1 day prior, when flecking was observed; in several instances, they were higher for 2 or 3 days prior to sporulation. In a series of experiments on $NiCl_2$, to be reported elsewhere, it was noted that ratios for diseased tissue were higher than for controls on the day of flecking. These latter results were obtained on tissue collected at least 5 hours after the start of the light period, in contrast to earlier collection times for the data of Tables 2 and 3. To establish whether time of collection with respect to onset of the light period was of critical importance, particularly at the time of flecking, the data of Table 6 were obtained. If the C_6/C_1 ratios for the morning collection of healthy and diseased plants are examined, they are found to be similar to data of Tables 2 and 3. During the flecking period, the diseased tissue ratio was, within error, identical to the healthy, even though the rate of respiration of diseased tissue was approximately 25% higher than the controls. On the morning of the 6th day, with 10% of the infections showing spore production, the ratio for diseased tissue was significantly lower than the controls.

In agreement with data from the nickel experiments, the afternoon diseased sample on the day of flecking showed a higher ratio than the healthy control. It is possible to question the significance of these results from the afternoon sample, because the difference resides in a drop in ratio for the healthy tissue from the previous and succeeding days. However, this phenomenon has been observed also in other experiments.

In the case of bean, the independence of respiratory rate and the decline in C_6/C_1 ratio of diseased plants have been consistent. Also, the change in C_6/C_1 ratio occurs when spores are being formed (Tables 4 and 5) with 1 significant exception (Fig. 2). In this instance, infected leaves showed flecking toward the end of the 3rd day but on the 4th day the ratios were still only slightly below those of the control plants. On the mornings of the 5th and 6th days, the ratios for diseased tissues were appreciably lower, but sporulation was not observed until the afternoon of the 6th day. It is important to note, however, that onset of sporulation in this experiment was extremely slow; complete (100%) sporulation was not observed until the 9th day. Because of the high infection intensities, the leaves were in poor condition and had to be discarded at this time.

These same data (Fig. 2) are valuable in that they show clearly a continuous rise in respiratory rate on the 3rd and 4th days with no significant drop in ratio. It is interesting that despite the difference in levels of

infection, both respiration rates and C_6/C_1 ratios for the 2 sets of diseased leaves are only slightly different. In the data of Table 3, a relatively light infection on trifoliate leaves resulted in higher ratios before flecks appeared. This has been observed in 1 other experiment on primary leaves with approximately the same level of infection but not in 3 other experiments. In all cases, however, the C_6/C_1 ratios at sporulation were significantly below the controls.

A 2nd aspect of the isotope experiments, which has been mentioned in the literature (1,18), is the effect of infection on percentage recovery of isotope from either the C_1 or C_6 positions of glucose. As explained in the section on materials and methods, the techniques and methods of calculation will determine appreciably the interpretation of data. With the techniques and calculations employed in this study, it was found that the lowering of the C_6/C_1 ratios in diseased tissue was associated primarily with a lower percentage recovery from the 6th position of glucose. The stage of development of the parasite, however, is critical in this respect. In most instances, the appearance of flecks signalled a lower percentage recovery for both the C_1 and C_6 labeled isotope when compared with healthy tissue. When spores first appeared, the C_6 recovery was most markedly repressed but when sporulation was well advanced the C_1 recovery of diseased tissue frequently was greater than the healthy controls.

Fig. 2 shows such a pattern, which is influenced somewhat by the level of infection. Heavier infection, during sporulation, led to higher percentage of recoveries for both C_1 and C_6 while lighter infection showed a rise in C_1, but a lower C_6 recovery during the same periods. The C_6/C_1 ratios were not significantly different.

Inhibitor studies.—The effect of $10^{-2}M$ sodium fluoride on healthy and rust-affected bean and wheat tissue was studied. In agreement with the data of Shaw and Samborski (17) with wheat, but in contrast to the results of Daly, Sayre, and Pazur (8), the effect was variable. With wheat during sporulation, when NaF inhibition should be most pronounced if a pentose pathway is operating cyclically, there was no difference in NaF sensitivity between healthy and diseased tissue. Two experiments with bean tissue gave opposite results; in 1 instance there was no difference, in a 2nd case respiration of diseased tissue was inhibited only 34% while that of healthy tissue was inhibited 68%.

Discussion.—The data presented in detail in the previous section are only a portion of a number of related studies. They are typical in demonstrating that infection by fungi causing rust diseases results in characteristic alterations in rates of respiration and a shift in metabolism which is indicated by a drop in C_6/C_1 ratios. These changes occur in susceptible host-parasite combinations irrespective of type of host tissue, age, environmental factors, and infection intensity. It is obviously impossible, in view of the large number of genetically specific host-parasite combinations known in rust diseases, to generalize with finality; but similar studies, with other rust species (8) and a different race of stem rust on the same wheat variety (17), suggest that these responses may be general in such infections.

Of more immediate importance, however, is the significance of the phenomenon in the development of those infections which have been studied in detail. Shaw and Samborski (17) concluded that the drop in C_6/C_1 ratio was inversely correlated with the increase in rate of respiration of diseased tissue. The basis for this conclusion is not an analysis of samples collected daily from reasonably uniform populations of healthy and diseased plants, but rather a graph of percentage increase in respiration plotted against the ratio of diseased to healthy C_6/C_1 values. From the relatively small number of points obtained at random from several different sets of plants, a regression line was calculated with a high P value. In addition to the absence of systematic data including any other possible variables which might correlate with C_6/C_1 values, a major criticism of such a technique is the assumption that the calculated regression line necessarily has a sound biological basis. Data in the present paper, when assembled in such a form, also yield a suitable regression line, but if individual experiments are examined, it is seen that respiratory rates were significantly above the rates of control plants for at least 24 hours before the decline in C_6/C_1 ratios.

Although a correlation can not be established statistically, we have observed that the drop in C_6/C_1 ratio occurs either when spores are first visible or shortly before. In most instances, a definite drop is observed only when sporulation is visible, even if only a small percentage of pustules with a limited number of spores in the center. With very heavily infected bean leaves, and slow reproductive development, it has been observed before sporulation but at least 24 hours after flecks are apparent (Fig. 2). Even in such cases, the respiratory rates had risen 24 hours before the drop in ratio. The uredial cycle of bean and wheat rust is completed in 6-8 days and the metabolic changes so far described are compressed into the last 3-4 days. To establish with precision the crucial period in which the change in C_6/C_1 ratio occurs will be difficult technically. It would require sampling at intervals of a few hours for at least three 24-hour periods. Further, the C_6/C_1 assay itself is time dependent, and, during the assay, development of the parasite presumably must be proceeding. There also are difficulties due to diurnal fluctuations in metabolism and the possibility of ontogenic metabolic variation of the host.

In any event, the present data clearly show that an abrupt shift in the type of respiratory metabolism occurs after the respiratory apparatus has been geared to a higher velocity, but whether this is directly related to, and required for, sporulation will be answered only after more basic information on the induction mechanism and on the other metabolic consequences of the decline in C_6/C_1 ratios is known. Data to be published by Daly and Jensen on the effects of inorganic nickel may be useful in such studies. $NiCl_2$

sprayed on rusted plants when in the fleck stage prevents normal spore production as described previously (13), and at the same time stops the sharp decline in C_6/C_1 ratio. However, respiration rates are as high or may be higher than unsprayed rusted wheat. The data of Silverman on the effect of temperature changes during late flecking and early sporulation also may be of significance in ascertaining the relationships between metabolic pathways and consequent development (19). In his work, it was shown that the greatest alteration in sporulation, or chlorosis and necrosis around the pustules, occurred when temperature shifts were made during the period in which we have noted the change in C_6/C_1 ratio.

In addition to the intrinsic biological importance that may be attached to the time of appearance of the decline in C_6/C_1, this information is necessary for detailed studies of the specific biochemical steps that are associated with the drop. In previous work, it has been assumed that a pentose pathway is responsible for the lowering of C_6/C_1 ratio, since it was the only pathway demonstrated in plants which would result in change in ratio, lack of NaF inhibition, and inhibition of the Pasteur effect.

The present data indicate that the Pasteur effect is not sufficiently consistent to be included in a detailed analysis of respiratory metabolism of rust-affected tissue. Although an inhibition of the effect can be observed in some instances of infection, its magnitude will be dependent on the technique employed in its measurement and to a considerable extent on the physiologic state of the host tissue. With wheat, $Q^{N}_{co_2}/Q_{co_2}$ ratios for diseased tissue were somewhat lower than for healthy tissue, while with infected bean, they were not; yet, the C_6/C_1 ratio indicated that similar changes in metabolism occurred in both species.

The effect of NaF does not appear suitable as a criterion for either the time at which metabolic transformations occur or the nature, necessarily, of shifts detected by other techniques. In both bean and wheat tissue, its effect is variable, even late in parasite development when maximum differences between healthy and diseased tissue would be expected. It is possible that the inhibition of respiration of rust-affected bean and wheat tissue, in contrast to the effect on safflower, may be due to differences among various host-parasite combinations in the extent of shunt metabolism or due to the level of infection. In foliar tissue, a limited bypass of glycolysis to the triose level may be operating (17), but it is not possible in the present studies to eliminate an inhibitory effect of NaF on enzyme systems other than enolase (8). Furthermore, the relative sizes of substrate and enzyme pools in healthy and diseased tissue may lead, because of the kinetic complexities, to erroneous concepts of pathways.

As an example, Farkas and Kiraly (9) have concluded that there is a metabolic modification in rusted tissue, presumably in Kreb's cycle activity, because they obtained less inhibition with malonate. Unpublished results with safflower and bean rust tissue also show less inhibition of respiration with malonate. The effect of malonate can be explained by the much higher levels of succinic acid that we have found in rusted tissue. Since malonate is a competitive inhibitor of succinic dehydrogenase, failure to obtain inhibition is more readily explained by substrate pool size than by invoking a new pathway.

The only supporting evidence that a pentose pathway occurs in the later stages of infection by rust fungi is the report (12) that radioactive sedoheptulose can be more readily detected in rusted tissue than in healthy tissue. Since carbon dioxide was the source of radioactive carbon, the data may, however, reflect increased turnover of sedoheptulose by anabolic reactions, rather than by respiratory catabolism. Although not yet demonstrated in fungi or higher plants, other pathways such as Entner Doudoroff might explain the drop in C_6/C_1 ratio in foliar infections. To avoid ambiguities in establishing a pathway, the relationships between rates and shift in C_6/C_1 ratios should be clearly established.

The transformation to a lower C_6/C_1 ratio occurs quite abruptly and thus offers an interesting kinetic situation. There are a number of triggering mechanisms which can be postulated and Shaw, *et al.* (18) have presented evidence implicating indoleacetic acid. It previously had been shown that IAA increases in both safflower and wheat tissue (6, 16). It was suggested that, in safflower, increases in hormones were responsible for the initial increases in respiratory metabolism by an indirect effect on growth (7), but hormones were not invoked in discussing the shift in types of pathway. Shaw's data showed that short exposures (1½ hours) to relatively high concentrations (1-10 mg/liter) of IAA caused a lower ratio in healthy wheat sections. Such rapid induction by IAA treatment is in contrast to other similar studies on intact plants with higher concentrations (11, 12). Shaw concluded, however, that IAA could not be involved in rusted tissue since the lower ratio in IAA-treated healthy wheat was due to a lower C_6 recovery, while in rusted wheat it was due to higher C_1 recovery.

Although calculated by different methods, selected as more representative of the kinetic realities, the data for bean and wheat show that rejection of the hypothesis concerning IAA is not valid. First, the recovery data for healthy tissue is variable, in some instances differences in ratio are due to either C_1 or C_6 or both. This is also true for treatment with hormones (11). Secondly, in our experience, rusted tissues show lower ratios usually because of a drop in C_6 recovery rather than C_1. This is particularly evident during the 1st 2 or 3 days after the drop in ratio has occurred. In later stages, depending on intensity of infection, C_1 recovery and sometimes C_6 will be above the controls. The data on recovery in the current study are in keeping with known accumulation of sugar and organic acids in initial stages of rust infection. Higher levels of respirable substrate in rusted tissue should lead to dilution of tagged carbon from exogenous glucose; but C_1, if preferentially metabolized, will yield rela-

tively more C_{14} when compared to the controls. This would explain the shape of the recovery in Fig. 2, since in the later stages of heavy infection the amounts of endogenous sugar decline.

Thus, it is not possible to eliminate an inducing mechanism only by a consideration of percentage of recoveries; the role of hormones should be reconsidered along with other possibilities. There are several reaction mechanisms, such as electron transport involving TPN and DPN, where interactions between multienzyme systems could occur. Couri and Racker (5) have evidence indicating that substrate competition between reconstructed glycolytic and pentose pathways may control the relative rates of the 2 systems. Beck (3) has suggested that the hexokinase reaction may be the key step in determining which pathway operates. An interesting case of inhibition of the glycolytic pathway by a substrate of the pentose pathway has been demonstrated by Parr (14). Although metabolic control is assumed in the above discussion, a change in physical properties of infected leaves might account for the sharp drop. For example, the protrusion of the spore-bearing layer results in rupture of the epidermis and may affect the partial pressures of O_2 and CO_2, thereby altering the rates of oxidative decarboxylation.

Although it is possible that spores are responsible for the apparent change in metabolism, there are several arguments against the idea. The tissue was washed thoroughly and the number of spores in relation to the total amount of tissue was negligible. As mentioned earlier, the drop in C_6/C_1 ratio occurred even with a trace of sporulation and in the experiment of Fig. 2 before any spores were observed. There has been no direct evidence, however, with *rust* diseases, as to the nature of the tissue, host or parasite, which is responsible for changes in respiratory metabolism. Fig. 2 suggests host tissue since the amount of infection does not greatly influence the quantitative data. Such evidence could be misleading; the number of infections does not necessarily measure the total amount of fungus cells present. We have frequently observed that the higher the intensity of infection, the smaller the pustule area, suggesting that competition among pustules restricted development.

The data suggest that several previous assumptions and conclusions concerning respiratory metabolism in obligate parasitism should be reconsidered. Some of the uncertainties reside in techniques employed. Some uncertainties can be resolved if critical attention is devoted to a rigorous analysis of the developmental stages in obligate parasitism. It will be necessary, if sound principles are to be obtained, to consider the physiologic aspects of infection as a sequence of metabolic steps which are compressed into a relatively short time. The sequence of steps may be influenced at least partially by diurnal fluctuations of the host, which in turn is undergoing developmental changes. In view of this, it is important that systematic studies with homogenous populations be conducted with attention to many complex variables, such as environment, status of host tissues, and levels of infection.

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SYMPTOMATOLOGY OF THE LEAF-CRUMPLE DISEASE IN SEVERAL SPECIES AND VARIETIES OF GOSSYPIUM AND VARIATION OF THE CAUSAL VIRUS

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SUMMARY

A severe strain of the leaf-crumple virus of Upland cotton (*Gossypium hirsutum* var. Acala 4-42) was transmitted by grafting but not by mechanical methods or by dodder. Mosaic, puckering, downward cupping of the leaves, and asymmetrical ruffling of the petals characterized the disease on 4 Upland cotton varieties. The variety Marie Galante appeared resistant. Plants of some *G. herbaceum* varieties were susceptible, but var. "Wightianum" was resistant. *G. thurberi* was severely affected by the virus, but plants of several varieties of *G. arboreum* were either immune or highly resistant. Symptoms on *G. barbadense* plants were similar to Acala 4-42 except that many affected leaves curled slightly upward, and were less severely puckered. Within *G. barbadense*, var. Domains Sakel was more severely affected than either Sakel L or Pima S-1. Yield of dry seed and lint was severely reduced by graft inoculation of 28-day-old Acala 4-42 plants but not of 42-or 56-day-old plants. In addition to the severe virus strain, a mild strain was also recognized. When plants infected with the mild strain were inoculated with the severe, mild symptoms persisted and only the mild strain was recovered.

INTRODUCTION.—The leaf-crumple disease on Upland cotton (*Gossypium hirsutum* L.) is caused by a virus transmitted either by grafting or by the white fly, *Bemisia tabaci* (Genn.), formerly designated as *B. inconspicua* (Quaint.) (2, 7). The leaf-curl disease of cotton in the Sudan is caused by a virus which is also transmitted by *B. tabaci* (5, 9). A new virus disease of cotton in Texas was described by Rosberg (8) in 1957, who considered it different from leaf-crumple because of vein banding and red interveinal spotting symptoms on young and old leaves which were not described for leaf-crumple. No insect vector has been reported for the Texas virus.

This study is an effort to further characterize the leaf-crumple virus by symptomatology on different *Gossypium* species, to search for a source of resistance, and to compare a mild and severe strain of the virus. Some of the results have been presented in an abstract (3).

METHODS.—Unless otherwise designated, the susceptible Upland Acala 4-42 variety of cotton was used as a host and test plant. Inoculum was collected in the Imperial and Coachella valleys of southern California and transmitted to healthy cotton plants by grafting. In some experiments 1-2 in. stem sections were grafted to the main stem of the suscept (side graft) and in others, an intact branch from the infected plant was grafted to the stem of the suscept (approach graft) and allowed to remain attached until symptoms appeared. In all varietal and pathogenicity tests inoculum from a strain which induced severe symptoms was used.

Greenhouse temperatures, varying from about 23°C at night to 35-37°C during the day, were favorable for the growth of cotton. Since plant growth in the greenhouse was much more rapid and less variable in a 50% sand-50% peat moss mixture than in soil, the U.C. mix "C,"supplemented with fertilizer "I" (1) and followed by weekly irrigations with a modified Hoagland's solution (4) was used for most tests.

RESULTS.—*Transmission.*—Mechanical inoculations were made to Acala 4-42 cotton, Scotia bean, Blackeye 5 cowpea, *Nicotiana glutinosa* L., and *N. tabacum* L. by rubbing expressed sap from leaves, flower petals, and lint from green bolls of infected cotton on Carborundum-dusted 1st true leaves of 10-20-day-old plants. In some tests, 1% solutions of K_2HPO_4 or Na_2SO_3 were added to the inoculum, and in others these compounds were sprayed on leaves just prior to inoculation. No infection resulted. Inoculation by Yarwood's fresh-leaf method (10) likewise failed to give infection.

Although dodder (*Cuscuta subinclusa* D.&H.) became established on both virus-infected and healthy plants, there was no transmission to Acala 4-42 cotton, kenaf (*Hibiscus canabinus* L.) or okra (*H. esculentus* L.).

Transmission was readily accomplished by side-grafting an infected stem section to the stem of a healthy plant or by excising a piece of bark from the

stem of a healthy plant, replacing it with bark from a diseased plant, and covering the wound with grafting tape. Transmission was also obtained when a root from each of 2 infected plants was grafted to roots of healthy plants and when root tissue from infected plants was grafted to stems of healthy plants. Infection was further verified by transmission from graft-inoculated plants back to healthy Acala 4-42 plants.

About 70-80% of the plants (less than 1 month old) inoculated by the side-grafting method showed symptoms 3-6 weeks after inoculation. Failure of inoculated plants to show symptoms was usually traceable to desiccation or disintegration of the inoculum tissue. Approach-grafting a branch from a diseased plant to a healthy plant consistently gave 95-100% infection, and symptoms usually appeared within 3 weeks. Although time for symptoms to appear was variable, they usually appeared sooner on younger plants. For example, in 1 experiment, symptoms on 1-month-old plants were seen 2-3 weeks after inoculation, but not on most 2½-month-old plants until 10-14 weeks after inoculation.

Infected cotton plants were approach-grafted to kenaf, okra, and hollyhock (*Althea rosea* Cav.) for as long as 3 months, but there was no evidence of either graft union or virus transmission.

Symptom expression on varieties and species.—To characterize the leaf-crumple virus by symptoms on different host plants and to search for sources of resistance, a study was made of the effect of the virus on several species and varieties of *Gossypium* in the greenhouse. Symptoms described were observed following graft inoculations of at least 4 plants/variety in each of several tests, after which the presence of the virus was verified by transmission back to healthy Acala 4-42 plants. Noninoculated plants were maintained as checks in each experiment.

1) *Gossypium hirsutum L.*—Affected leaves from diseased plants of the varieties Acala 4-42, Delta Pine, Glandless, and an experimental variety, R15 (U. S. Department of Agriculture, Brawley, Calif.), were cupped downward, distinctly more puckered and crinkled than healthy leaves (Fig. 1-A, 4). The leaf-veins were distorted, thickened, and often cleared when viewed with transmitted light (similar to that shown for *G. thurberi*, Fig. 2).

On rapidly growing plants, mosaic symptoms were usually present. The leaf color of plants that had been infected for long periods in the greenhouse was usually more intense blue-green than normal. On senescent leaves, reddish spots within chlorotic interveinal areas and green veinbanding were occasionally noted. These symptoms bear some resemblance to those described by Rosberg (8) except that he also noted reddish spots on young leaves.

On plants allowed to recover after pruning, shortening and thickening of the internodes induced a "bunchy" appearance of the leaves, especially on plants inoculated with a severe strain of the virus (Fig. 4-C). When inoculated plants were not pruned back, stunting of infected plants was seldom noted; and, in many cases, especially in the field, infected plants were slightly taller than healthy plants.

Infected flowers (Fig. 1-B) were asymmetrically puckered and ruffled, and the many protuberances on the petals gave them a crinkled appearance. In the field, flower symptoms appeared distinctive and unlikely to be confused with other effects, whereas crinkled leaves sometimes might be confused with distortion caused by insects. Infected bracts at the base of the boll commonly became asymmetrical, veins were swollen and twisted, and the bolls more pointed and bumpy than on healthy plants (Fig. 1-C).

Since the var. Marie Galante is considered resistant to the leaf-curl virus in the Sudan (5), the reaction of this variety to leaf-crumple was tested. When 5 plants of a source designated Texas 371 were inoculated by side grafting, no symptoms appeared after 9 months. The plants were then reinoculated in the same way, and 3 months later, although the infected Acala 4-42 scions were still viable, only 1 Texas 371 plant showed faint mosaic in the tip leaves. When the inoculated plants were tested for presence of virus by grafting to healthy Acala 4-42 plants, virus was detected in the plant showing mild symptoms and in only 1 other, which showed no symptoms.

In a repeat experiment approach graft-inoculated Texas 371 plants showed no symptoms after 5 months compared with Acala 4-42 plants which showed symptoms after 1 month.

2) *G. herbaceum L.*—When 4 plants from each of the seed lots C.B. 2939 (from Korea), C.B. 2940, and C.B. 2941 (both from Iran) were inoculated by side grafting, symptoms developed similar in severity and type to those on Acala 4-42. Plants of the var. "Wightianum" (Texas 435) appeared highly resistant since none of 10 inoculated plants (4 by side graft and 6 by approach graft) developed symptoms after 5 months and the virus was recovered from only 1 of them.

3) *G. arboreum L.*—When 5 plants of Texas 439, 440, 446, and 449, C.B. 2698, and 2700 (all from Korea) were inoculated by side grafting, no symptoms appeared after 10 months. Although on many symptomless plants the infected Acala 4-42 scion continued to grow, the virus was recovered from only 1 plant of C.B. 2700 by approach grafting to healthy Acala 4-42 plants. It appeared that plants of these varieties were either immune or highly resistant.

4) *G. thurberi Todaro.*—Four of 7 plants inoculated by approach grafting showed symptoms of the disease 1½ months after inoculation. The symptoms (Fig. 2) differed from Acala 4-42 in that leaves were not severely puckered or cupped and tended to become more asymmetrical and twisted. Vein thickening and clearing and leaf mosaic occurring in diffuse chlorotic spots 7-10 mm in diameter were characteristic.

5) *G. barbadense L.*—Since *G. barbadense* is grown in the Sudan where the leaf-curl virus disease of cotton occurs (5, 6, 9), inoculation of varieties of that species with leaf-crumple permitted an indirect comparison

A
B
C

←

Fig. 1. Symptoms of the leaf-crumple virus disease on *Gossypium hirsutum* var. Acala 4-42. Left, from a healthy plant; right, from an infected plant. **(A)** Leaves. **(B)** Flowers. **(C)** Bracts and bolls.

of the 2 diseases. Var. Pima S-1 and 2 varieties grown in the Sudan, C.B. 3033 (Sakel L or X 1730A), resistant to the leaf curl virus (9), and C.B. 3032 (Domains Sakel), a susceptible variety, were studied. In 1 experiment when 4 plants (5 weeks old) of these varieties and Acala 4-42 were inoculated by side-grafting, symptoms occurred 3 weeks later on Acala 4-42, but not until 10 weeks later on Sakel L and Domains Sakel, and 15 weeks later on Pima S-1. In 4 subsequent tests in which inoculation was by approach graft, symptoms occurred on the *G. barbadense* varieties in 5-9 weeks.

Symptoms on the *G. barbadense* varieties were much milder than on Acala 4-42. The Domains Sakel variety was the most susceptible, the leaves being small, asymmetrical, puckered, and tending to cup upward with veins thickened (Fig. 3). On some severely affected leaves, enation-like papillae not over 1-2 mm in length appeared on distorted veins. With transmitted light the veins on affected leaves were usually cleared. A mosaic was often present. In contrast, Sakel L was less severely affected (Fig. 3), and occasionally a symptomless branch, from which the virus could not be recovered, appeared on infected plants. Pima S-1 was even less severely affected.

Variation of the leaf-crumple virus.—In a collection of Acala 4-42 plants, each inoculated from a different field source, some appeared to be more severely affected than others. To determine if different strains of the virus existed, a plant inoculated with the severe strain, and a plant showing similar but milder symptoms were compared as inoculum sources. After 1 month, symptoms were more subdued on the 5 Acala 4-42 plants inoculated with the mild source, in some cases, than on the 5 plants inoculated with the severe source. In other cases, differences in initial symptom expression were difficult to distinguish. However, after pruning inoculated plants, consistent and striking differences appeared on the regrowth 3 months later (Fig. 4). The average height (3 months after pruning) of the plants inoculated with the mild isolate was 31 cm, compared to only 12 cm for those inoculated with the severe isolate. These data were typical of 2 additional experiments.

Cross-protection was shown by the mild virus strain against the severe. When plants with mild symptoms were inoculated with the severe strain, the mild symptoms persisted 6 months later. Only the mild type was recovered when healthy plants were inoculated by approach grafting. Two months after pruning the average of oven-dry weights of petioles and leaves from uninoculated plants was 4.6 g; from plants inoculated with the mild strain, 2.5 g; from plants inoculated with the severe strain following the mild strain, 2.8 g; and with the severe strain, 0.9 g.

The relative proportion of these strains in the field is not known, but may be an important epidemiological factor.

Effect of inoculating plants of different ages on boll set and yield of cotton.—In a field test, natural infection of plants during midsummer reduced numbers of bolls and weight of seed cotton, whereas natural infection in late summer had little effect on yield (3). Such a phenomenon might be of epidemiological importance.

Plants grown in 9-in. clay pots were inoculated by approach-grafting 28, 42, and 56 days after planting. Inoculated plants were not detached from the virus source plants until symptoms appeared. Control plants

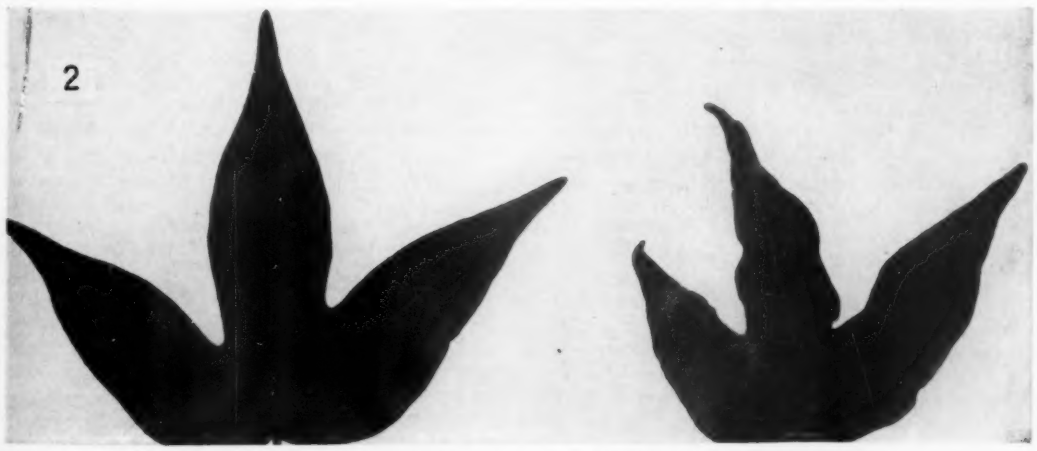

Fig. 2. Symptoms of leaf-crumple in *G. thurberi.* Left, uninoculated leaf; right, inoculated.

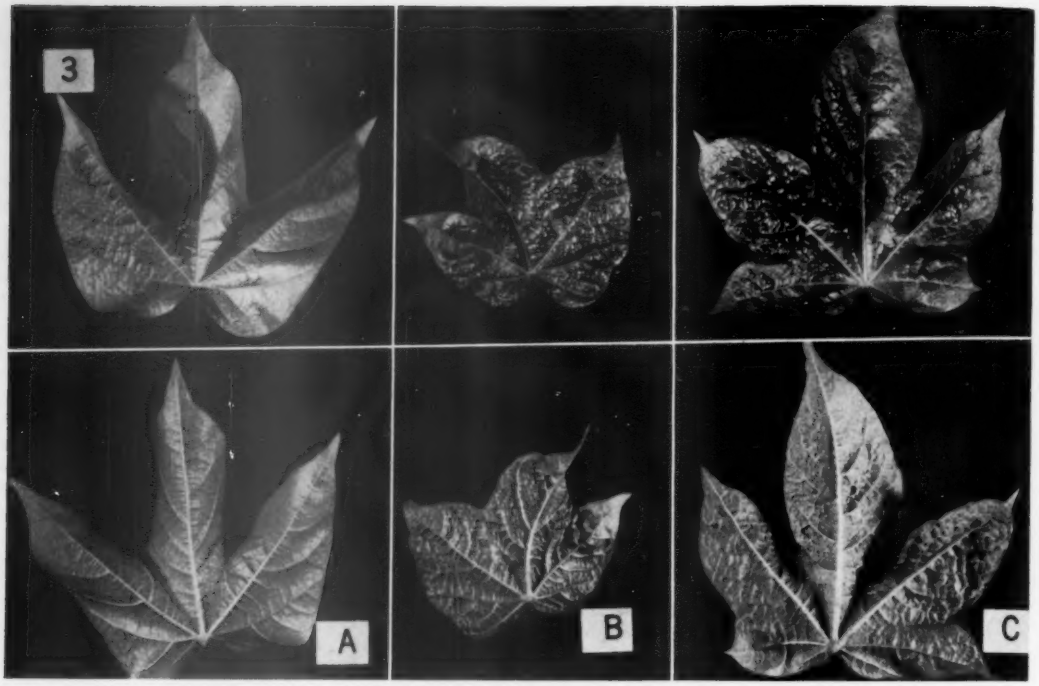

Fig. 3. Symptoms of leaf-crumple on *G. barbadense*. **(A)** Upper and lower sides of uninoculated leaf of var. Domains Sakel. **(B)** Inoculated Domains Sakel. **(C)** Inoculated Sakel L. (Leaves of uninoculated plants of both varieties were similar in size and shape.)

were detached from their healthy counterparts on the date the first inoculated plant showed symptoms.

The average numbers and weights of bolls and weights of plants (Table 1) showed that the lowest yield resulted from inoculation of young 28-day-old plants. Although the yield of mature cotton bolls on older inoculated plants was not appreciably reduced, the numbers of green bolls were considerably reduced at the termination of the experiment. Symptoms appeared more rapidly following inoculation of young plants than older plants. At the termination of the test, average dry weight of the plants inoculated at 28 days was slightly greater (143 g) than that of the noninoculated checks (118 g). This may have been due to the setting of fewer bolls by infected plants and their tendency to remain in a vegetative state. In the field, infected plants with a light set of bolls are occasionally slightly taller than noninfected plants.

Since the weight of cotton seed and lint was reduced by inoculation of 28-day-old plants but not 42- or 56-day-old plants (Table 1), younger plants may be more susceptible to the virus. However, the striking reduction in numbers and dry weights of green bolls at the termination of the experiment on plants inoculated at all ages suggested that the effect of the virus might merely have been delayed in late inoculated plants.

Discussion.—Laird and Dickson (7) pointed out the similarity in the virus-vector relationship of leaf-curl (5, 9) to leaf-crumple and noted that the leaf-curl susceptible *G. barbadense* var. Domains Sakel was also susceptible to leaf-crumple. They concluded that symptoms of leaf-crumple on Domains Sakel were quite distinct from published descriptions (6) of the leaf-curl virus. For example, we have noted that leaf-crumple virus appears to induce more crumpling and puckering of leaves than leaf-curl. Although both

Table 1. The effect of inoculating plants of different ages with leaf-crumple virus on the numbers of bolls and dry weight of cotton seed and lint produced

Age of plant at inoculation (days)	Time for symptoms to appear (days-range)	Mature bolls set (no.)	Dry weight at termination of experiment		
			Cotton seed and lint (g)	Green bolls[a] (no.)	Green bolls[a] (g)
28 I[b]	14–46	8.5	26	2.2	6.5
28 NI[c]		14.0	61	6.0	9.5
42 I	30–95	11.3	51	3.8	4.2
42 NI		15.5	60	10.0	17.0
56 I	72–134	12.0	50	4.8	6.0
56 NI		13.5	54	10.5	24.2

[a] Oven-dried weights 245 days after planting.
[b] I = Inoculated.
[c] NI = Noninoculated controls.

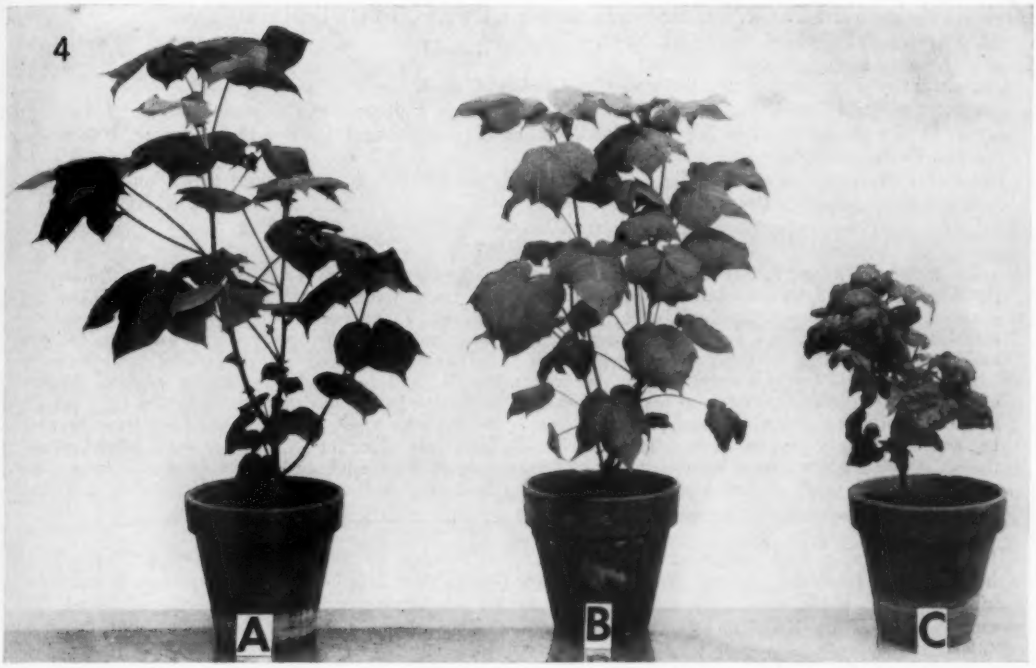

Fig. 4. Comparison of the effect of 2 virus strains on Acala 4-42 cotton. **(A)** Uninoculated. **(B)** Inoculated with a mild strain of leaf-crumple virus. **(C)** With a severe strain. (Photographed 2 months after the plants had been uniformly pruned.)

viruses cause vein thickening, veins on leaf-crumple affected leaves are more tortuous, and "cup-shaped" enations of leaf-curl are absent (9). Leaf-crumple causes vein clearing but not the "vein clogging" (9) attributed to leaf-curl. In general, symptoms of leaf-crumple appeared milder on *G. barbadense* than on *G. hirsutum*, whereas leaf-curl is considered more severe on *G. barbadense* (5, 9). *G. herbaceum* varieties with the exception of "Wightianum" were susceptible to the leaf-crumple virus, but certain unspecified varieties of this species were reported immune to leaf-curl in the Sudan (5, 9). Also, leaf-crumple virus causes a distinctive crinkling of flowers in contrast to blasting of flowers reported for leaf-curl (9).

Despite these differences, the similar vector relationship (7), tolerant or resistant reaction of Marie Galante, the relatively mild reaction of Sakel L, and apparent immunity of *G. arboreum* varieties to both viruses, suggest the possibility that these 2 viruses might be related strains. In this study, differences in virulence between a severe and a mild strain of leaf-crumple virus were quite marked. Tarr (9) has suggested that more than 1 strain of leaf-curl might exist in Africa.

Resistance to the virus in the varieties Marie Galante or "Wightianum" might be of use as possible sources of resistance to leaf-crumple should a breeding program be warranted. Since only a few varieties of cotton were tested for resistance, undoubtedly many other resistant sources exist. Selection of plants resistant to leaf-curl has been highly successful (5). Illustration and description of the symptomatology on several varieties and species of *Gossypium* should assist in the identification of the leaf-crumple virus if it occurs in other areas.

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THE ORCHARD DEVELOPMENT OF PEACH POWDERY MILDEW

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SUMMARY

Sphaerotheca pannosa, the causal organism of peach powdery mildew, overwinters as mycelium in dormant buds. Shoots from buds containing the mycelium are infected as they emerge in the spring and provide inoculum for current season infection.

Infection of new leaves occurred throughout most of the growing season. The 1st current season infection appeared about 1 month after blossoming and the disease progressed rapidly until essentially all apical shoots were mildewed. The fruits were susceptible only during the early stages of their development. Two months after blossoming the fruits were completely resistant to mildew infection. Transition of fruit from mildew susceptibility to resistance occurred just prior to the pit hardening stage of growth which was between April 22-29 in the 1957 growing season. Lesion extension by mycelial growth continued for a period of 2 weeks after the fruits were resistant to new infections. Neither foliage nor fruit infection appeared to be influenced by environmental conditions in the orchard.

INTRODUCTION.—The causal organism of this disease is the fungus *Sphaerotheca pannosa* (Wallr.) Lev. var. *persicae* Wor. However, essentially all reports of *S. pannosa* on peach are based on observations of the conidial stage (*Oidium leucoconium* Desm.) due to the rare occurrence of cleistothecia. The disease appears in the same orchards year after year and in 1894 Smith (6), on the basis of early infected shoots and the absence of any other source of inoculum, suggested that the fungus overwinters in buds. Yarwood (10) suggested that peach and rose mildew overwinter in buds because infected shoots emerged from twigs held in test tubes while the buds opened. However, he did not demonstrate the location of the mycelium within the buds.

Certain observations have been made on the development of peach powdery mildew in the orchard. Whipple (8) in 1906 stated that *S. pannosa* thrives best in a warm, moist, and shaded location, and that sunlight is its worst enemy. Stewart (7, p. 221) stated that, in the nursery, peach mildew is usually prevalent only when there is an excessive amount of moisture. Fikry observed (3) that *S. pannosa* appeared when the water table and air humidity were lowest. In a later publication, Fikry (4) stated that severity of attack of peach by the fungus is favored by close planting of trees. Louw (5) in 1946 reported that conditions of high humidity accompanied by high temperature favor infection of peach by mildew, whereas the presence of free moisture retards it.

The principal economic loss from powdery mildew of peach results from fruit infection which, if severe, renders the fruit unacceptable to the canner. This study was directed toward determining the factors which influence the severity of fruit infection, including overwintering of the fungus, foliage infection, inoculum build-up, and the period during their development when fruits are susceptible to infection.

The present study was conducted primarily in 1957 on 25 trees in a 37-year-old Paloro peach orchard that in previous years had been severely mildewed. These trees were omitted when any routine control measures were applied to the orchard. Because tree and fruit growth may vary from season to season, it was necessary to relate disease development to host development rather than to calendar dates. The growth curve of the fruit was used as an index of host development. Continuous temperature and relative humidity records were made to determine the influence of environment on disease development.

Overwintering of the fungus.—This work was conducted to further verify the presence of viable fungus within dormant buds and to determine the location of the mycelium. Dormant twigs, from mildew-diseased trees, were brought to the laboratory, wiped with a cloth soaked in sodium hypochlorite (1:10 dilution of Purex) and placed in tubes containing 20 ml of water. The tubes, each containing a single twig, were covered with a double layer of cheesecloth, secured with a rubber band, and held at 72±4°F until shoots began to emerge. When green tips of the leaves extended 3-5 mm beyond the bud scales, buds were dissected under a binocular dissecting microscope. Each bud scale was removed individually and those suspected of bearing mycelium were cleared in alcohol-acetic acid, stained with lacto-fuchsin (1), and examined for characteristic signs of powdery mildew.

Fifty buds were dissected. Powdery mildew mycelium and conidiophores were identified on inner bud scales in 4 cases. Each time mildew was observed, mycelium was found on the outer surface of the innermost bud scale. Numerous twigs were held with no application of hypochlorite and there was no evidence that twig lesions from the previous year's infection contained any viable mycelium capable of sporulating.

In 1956 twigs were collected at different times dur-

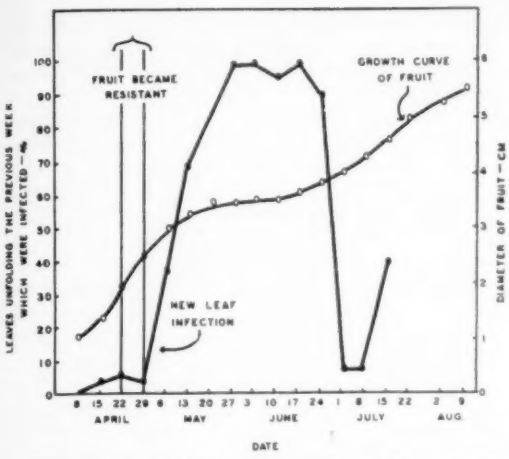

Fig. 1. Long period of powdery mildew build-up on new leaves; 7-day period in April during which fruit became resistant to infection and which separated a short period of susceptibility from the remainder of the season when fruit was completely resistant; growth curve of fruit of Paloro peach in 1957 which can be separated into 3 stages: Stage 1, April 8 to May 20; Stage 2, May 20 to June 17; and Stage 3, June 17 to August 9.

ing tree dormancy and handled as described above. As the dormancy period progressed, the percentage of infected new shoots decreased. Of 60 twigs collected January 6, 1956, 42 apical shoots emerged, of which 24% were infected. Of 200 twigs collected February 16, 1956, 171 produced apical shoots of which 17% were infected and of 99 apical shoots emerging from 100 twigs collected March 2, 1956, only 9% were diseased. Most of the infected shoots were from apical buds and only rarely were lateral shoots infected.

Growth curve of the fruit.—The cumulative growth of the fruit (Fig. 1) was obtained from weekly diameter measurements at the suture, of fruits selected at random. Fifty fruits were selected before jacket fall; at the end of the season, 36 of these fruits remained. Measurements, made with a vernier caliper reading to 0.1 mm, were started at jacket fall and continued to 1 week before harvest.

Growth of peach fruit can be divided into 3 stages (2) as follows: Stage 1, rapid development (April 8 to May 20; Fig. 1); Stage 2, rest period (May 20 to June 17, Fig. 1); Stage 3, period of rapid growth (June 17 to August 9, Fig. 1).

Fruit susceptibility.—The period of growth during which Paloro fruits were susceptible to infection by the powdery mildew fungus was determined in the orchard plot by 3 separate methods. One procedure was to protect fruits for various periods of time and then expose them to natural inoculum in the orchard. Fruits were protected by flannel sleeves, which were slipped over the end of the branches and secured at both ends with wire plant ties. In preliminary laboratory experiments, flannel was found to be effective in preventing conidial passage and this effectiveness was not changed by wetting and drying the material.

Three hundred branches distributed among 9 trees located in a group at the center of the plot were bagged on March 2, 1957, when the trees were at one-third full bloom. Beginning on March 19, and at weekly intervals for 14 weeks thereafter, 5 bags were removed from each of 4 trees, exposing the fruit to natural inoculum. At the end of the season, data were taken on the number of lesions which developed on fruit covered for varying periods of the growing season. Pollination occurred and fruit appeared to develop normally under bags but the leaves became somewhat chlorotic where the bags were left for long periods.

The bagging experiment indicated that fruits were susceptible up until April 15, but resistant on and after April 22 (Table 1).

A 2nd approach was to make weekly inoculations of fruits in the orchard. Small marked areas on 20 fruits were inoculated each week and observations for lesions made at weekly intervals. Fruits were susceptible when inocluated on April 15 and 22, but resistant to infection by April 29. These inoculations also provided data on the incubation period of the mildew on fruit (i.e., the period from inoculation to appearance of visible disease). This period was approximately 10 days. Lesions were not visible 1 week after inoculation, but after 2 weeks were 1-2 mm in diameter. Numerous attempts were made to infect fruits maintained under various conditions in the laboratory, but in no case did lesions develop on these fruits.

A 3rd method used to study fruit susceptibility was based on observations of fruit on 50 branches. The branches selected were distributed among 10 trees within the plot. Each week the number of fruits on each branch and the number of lesions on these fruits were recorded. These observations were begun on April 8 and at this time no mildew lesions were present on 300 fruits. Five lesions were noted on April 15 and 9 on April 22. By April 29 the number of fruits under observation had dropped to 254, and 41 lesions were counted. On May 6 there were 82 lesions and on

TABLE 1. Infection of fruit exposed to natural inoculum at 1-week intervals—fruit was covered from flowering until bags were removed

Date bags removed (1957)	No. of fruits exposed	Total no. of lesions
March 19	78	12
March 25	33	10
April 1	38	9
April 8	49	7
April 15	54	20
April 22	47	0
April 29	47	0
May 6	27	0
May 13	34	0
May 20	29	0
May 27	13	0
June 3	26	0
June 10	25	0
June 17	37	0

May 13, 97 lesions on 231 fruits. From this time until harvest the number of fruits dropped to 203, but no new mildew lesions were observed. On the basis that 10 days to 2 weeks are required from inoculation to appearance of visible lesions, the 1st infections must have occurred during the 1st week in April and the last infections during the period of April 22-29. The lack of fruit infection before the 1st week in April occurred probably because fruits were covered by the floral envelope until about April 8 and because of the small number of inoculum centers present at that time. It is possible, however, that fruits are not susceptible to infection earlier in their development since some inoculum was available from the middle of March.

Data from all 3 methods were in agreement that in 1957 fruits were resistant to infection by the end of April. The change from susceptibility to resistance to powdery mildew occurred during the middle of the 1st of the 3 stages of the growth curve (Fig. 1) at which time the fruits were approximately 2.5 cm in diameter.

Observations made during the 1958 season in the same plot gave strikingly similar results. Fruits were susceptible to inoculation on April 24 and resistant on May 1. Observations of natural build-up of infection substantiated the conclusion that fruits were resistant by May 1. As in 1957, the transition occurred during the middle of the 1st growth curve stage.

Relation of lesion extension to fruit susceptibility.—To determine the relation between the susceptibility of fruit to infection and the growth of established mycelium, the diameter of 25 lesions was measured on May 1. Repeated measurements were made at 1- or 2-week intervals for the following 8 weeks. Measurements were not started prior to this date because of the scarcity of lesions.

Mycelial growth as reflected by lesion extensions proceeded rapidly for 2 weeks after fruit was resistant to spore infection. On May 1, the mean diameter of 25 lesions was 0.29 cm, but by May 14 the mean diameter had increased to 0.50 cm. By May 27 the diameter had reached 0.58 cm and mycelial growth had ceased since no further increase in lesion size was observed.

Disease development on the foliage.—Mildew development on foliage was determined by weekly observations of 50 elongating apical shoots. The number of leaves which had unfolded the previous week and the number of these leaves showing visible mildew infection was recorded for each of the shoots (Fig. 1). Consideration was given to new shoot growth because only young leaves are susceptible to infection (8). This was verified in the present study on the basis of orchard observations, inoculation in detached leaf culture of leaves from orchard trees, and inoculation of seedling trees in the greenhouse. On orchard trees, leaves below the 2nd node from the shoot apex were resistant to infection.

By the middle of March, leaf buds had opened and shoots were expanding. Artificial inoculation of these 1st leaves showed that they were only slightly susceptible to *S. pannosa.* At this time mildew inoculum was present on shoots from buds in which the fungus had overwintered. There was no appreciable shoot elongation until the 1st week in April, when apical leaves became highly susceptible to artificial inoculation in detached leaf culture. There was no sign of current season infection (i.e., mildew on shoots other than those which emerged infected) until April 15. The lag between the presence of inoculum due to holdover mycelium and the occurrence of current season infection may have been due to resistance of the leaves until apical extension began or to a necessity for infected shoot expansion and consequent inoculum build-up.

There was a progressive increase in the amount of mildew on the foliage after April 15 until the leaves on essentially every growing shoot were infected. The leaves of many of the shoots were completely covered with mycelium while others had only scattered colonies of mildew. Growth of severely infected shoots was inhibited. This condition of essentially 100% infection of new growth continued for several weeks, followed by a sharp decrease in new infection.

Sulfur dust, applied to the surrounding trees in the orchard between June 17 and 24, may have drifted to the experimental plot and contributed to the sharp decrease in mildew infection. Also, the decrease could have been due to the high temperature during the 5 days June 23-27, when the maximum each day was above 100°F, rising to 108°F on June 24. Shoot elongation had almost ceased by July 8 and had completely stopped by July 15. Growth reduction may have occurred during June 24 to July 1 which would influence the susceptibility of the foliage.

Influence of temperature and relative humidity in the orchard.—Continuous temperature and relative humidity records were obtained with a Bendix-Friez, Model 594, hygrothermograph situated in a regulation weather station. The accuracy of the instrument was checked each week with a Friez, Model HA-2, hand bulb psychrometer. Hygrothermograph and psychrometer readings of relative humidity consistently agreed within 4%. Temperature agreement was within 2°F.

The values are presented on a weekly basis (Fig. 2) and can be considered as only a relative indication of the true situation. The mean weekly high and low temperatures were obtained by averaging the maximum and minimum temperatures of each day. Relative humidity followed a rhythmic daily pattern, increasing at night to saturation or near saturation and decreasing during the day. There was considerable variation, however, in the rate of increase and decrease and thus the length of time that relative humidity remained high or low. It was considered that the number of hours each day during which the air was at or above 98% relative humidity might be a satisfactory indication of moisture relations in the orchard.

Environment during the period April 29-June 17 appeared to be favorable for mildew infection of

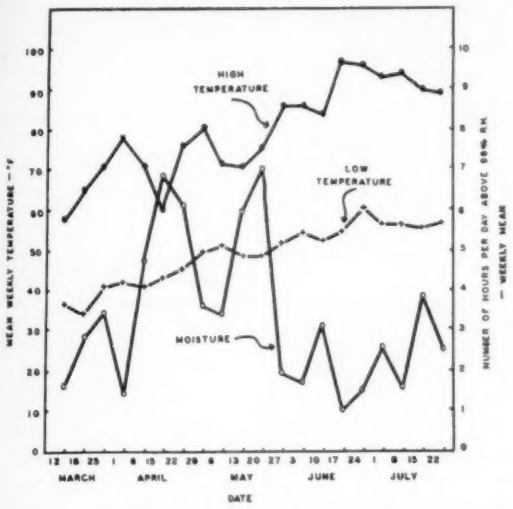

Fig. 2. Temperature and moisture conditions in the orchard during the growing season of 1957. April 22-29 marked the transition of the fruit from susceptible to infection to resistant. From April 29 to June 24 there was abundant infection of new leaves which did not appear to be influenced by the fluctuating environment during this period.

foliage as evidenced by the continuous and abundant attack of new growth (Fig. 1). During this time there were very noticeable fluctuations in both temperature and moisture conditions in the orchard (Fig. 2). Moisture variation was most striking, ranging from 2-7 hours a day above 98% relative humidity. Therefore, it is concluded that infection and development of powdery mildew on peach foliage can take place satisfactorily over a wide range of environmental conditions. This conclusion is strengthened by data (to be published later) on the temperature and moisture requirements for conidial germination. In this study it was found that germination was satisfactory over the temperature range of 15-30°C and that germination on the leaf surface was not influenced by atmospheric moisture conditions between 100 and 48% relative humidity at 25°C.

DISCUSSION.—The demonstration that viable mildew mycelium was present in dormant buds is in agreement with the conclusions of other workers (9, 6). The observation that viable mycelium was found only on the innermost bud scales and that fewer infected shoots were obtained from buds collected late in the dormant period indicates that mycelium must be deep within the bud in order to survive. Infected shoots arising from dormant buds provide inoculum for subsequent secondary or spore infection and disease development on foliage and fruit. The pattern of disease increase observed on foliage can probably best be interpreted as due to progressive increase in inoculum.

The data clearly show that peach fruits became resistant to mildew infection relatively early in their development. A similar relationship was observed in apricots by Yarwood (10). The pattern of susceptibility and resistance is the same for both fruit and foliage. In both cases young tissue is susceptible to mildew infection and as development and maturation progress a transition to complete resistance takes place. Because new leaves are continually being formed, mildew can develop on foliage throughout the growing season. Inoculum increase takes place on foliage and the severity of fruit infection is directly related to the amount of inoculum available during the period when fruits are susceptible. Any conditions which delay the increase of inoculum or shorten the period of fruit susceptibility would be expected to reduce the amount of fruit infection. In 1957 fruit infection was moderately severe and the fruits were resistant before the rapid increase of foliage infection (Fig. 1). In 1958, scant foliage infection occurred while the fruits were susceptible and fruit infection in the plot was negligible. It would be expected that the number of primary inoculum centers would influence the rapidity of inoculum build-up. For this reason effective control of foliage infection should result in fewer infected buds and perhaps less fruit infection the following season. The effect of environment on generation time of the fungus would also influence inoculum increase.

The transition of fruit from mildew susceptibility to resistance apparently is not the result of any direct effect of environment as conditions were favorable for leaf infection long after fruit had become resistant. Enlargement of mildew lesions for 2-3 weeks after fruits were resistant to new infections indicates that once the fungus is established it can overcome the defense mechanism of the host for a limited time.

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RELATION OF PREVIOUS NUTRITION OF GLADIOLUS TO INFECTION BY STROMATINIA GLADIOLI

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SUMMARY

Corm infection by *Stromatinia gladioli* was definitely influenced by previous nutrition. Varieties reacted differently to 16 combinations of high and low N, P, K, and Mg tested. October Sunshine and Spotlight generally gave similar results, but Spic and Span was often different. Increasing the levels of either magnesium or nitrogen increased the susceptibility of corms to infection, whereas increasing potash tended to decrease susceptibility slightly. The effect of phosphorus was variable. Several 1st order interactions were present, as well as some of the 2nd order. Magnesium was involved in many of these. Changing the potash level did not affect results with magnesium as much as did changing phosphorus. In general, it appeared that ratios of P and K should be balanced—either both high or both low. The results indicate that the cumulative effect of proper nutrition might be an important factor in controlling this disease.

INTRODUCTION.—Accumulating evidence indicates that the nutrition of bulbs influence their infection by pathogenic fungi. Thus, McClellan and Stuart (5, 6, 9, 10, 11) showed that: 1) high amounts of nitrogen increased losses from *Fusarium* rots of gladiolus and narcissus, and 2) the loss varied with the source of nitrogen. These same workers (6) also showed that gladiolus fertilized with low levels of nutrients are less susceptible to *Curvularia* and *Botrytis* leaf spots than are gladiolus fertilized with high amounts. Gould (4, working with Karl E. Baur) found, in contrast, in western Washington that fertilizers applied to soil infested with *Stromatinia gladioli* (Drayton) Whetzel had only a slight effect on the disease development of gladiolus during the same season.

Since gladiolus corms contain high reserves of food (6, 11, 12), it was suspected that their previous nutritional history might be more important than current nutrition in influencing infection by the dry rot fungus in western Washington. The following 2 experiments, therefore, were conducted to test the possible effect on disease resistance of corms fertilized previously with various combinations of high and low levels of nitrogen, phosphorus, potassium, and magnesium.

MATERIALS AND METHODS.—*1956 experiment.*—Picardy corms used in the 1956 experiment were grown from cormels planted in sub-irrigated beds of gravel in a greenhouse on February 16, 1954, at the Plant Industry Station in Beltsville. The solutions were changed at regular intervals. Low and high levels of N, P, K, and Mg were used in all 16 possible combinations. The low and high levels in ppm were: nitrate nitrogen, 14 and 70; ammonia nitrogen, 2.8 and 14.0; phosphorus, 7.8 and 38.8; potassium, 15.6 and 78.2; and magnesium, 12 and 60. The sources were: $Ca(NO_3)_2$, K_2SO_4, KCl, $(NH_4)_2SO_4$, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$. Minor elements (Fe, Cu, Zn, Mn, Bo) were added to all solutions. The corms produced varied considerably in size (from No. 2 to 4) because of different growth responses to the various nutrients. The results with these corms, however, indicated little or no relation of size to infection by *Stromatinia*. The corms were planted May 10, 1956, in an artificially infested Puyallup sandy loam soil contained in tiles (3) at the Western Washington Experiment Station. Three replications of 100 corms were used for each nutrient type. Corms were harvested on September 28, cured, and cleaned, and data were obtained on October 12 on amount of disease, size, and weight.

1957 experiment.—Corms used in the 1957 experiment were grown in 1956 at Beltsville in solutions similar to those in 1954, except that levels of nutrients were slightly different: 7 and 70 ppm of nitrate nitrogen; 1.4 and 7 of ammonia nitrogen; 3.9 and 38.8 of phosphorus; 14.8 and 78.2 of potash; and 12 and 60 of magnesium for low and high levels, respectively. The corms were stored at Beltsville during the winter of 1956-57 at 40° F and a relative humidity of 60-70%. They were planted on May 2, 1957, at Puyallup, in a field of Puyallup loam soil. The loam had a pH of 5.7 and was low in phosphorus, magnesium, and calcium; medium in nitrogen and potash; and very low in chloride and sulfates. At planting, oat inoculum of the dry rot fungus was distributed uniformly in the rows at a rate of 6 cc/ft. Five replications of 3 varieties were arranged in a randomized block. The number of corms of various sizes of each variety used are shown in Table 1.

Overhead irrigation was used to supplement rainfall, whenever necessary. Corms were harvested on September 25, cured, and cleaned, with all husks removed for accurate estimation of the area infected

TABLE 1. Numbers and sizes of corms used/variety and replication, and the length of row/variety in 1957

Variety	No. of corms/size/replication (cm circumference)						No. of ft of row/ replication
	4-5	5-6	6-7	7-8	Above 8	Total	
October Sunshine	25	10	3	2	3	43	9
Spic and Span	15	3	1	2	1	22	4.5
Spotlight	10	4	1	3	1	19	4.5

with dry rot. The weather in 1957 was unusually favorable for development of the disease. As a result, almost all corms had at least 1 lesion, but there were noticeable differences between lots in degree of infection. The corms were therefore sorted into 7 disease index classes: 6, healthy; 5, 0-20% of the area covered with dry rot lesions; 4, 21-40%; 3, 41-60%; 2, 61-80%; 1, 81-100%; and 0, corms missing, presumably entirely rotted. Thus, indices of 1 and 2 represent respective averages of about 90% and 70% of the corm surfaces covered with dry rot lesions.

RESULTS.—*1956 experiment.*—The most important criterion of the effect of nutrition was the production of healthy corms. Although differences in the data were not significant, certain trends were apparent. Thus, most healthy corms were produced from parent corms with a previous history of low nitrogen and low phosphorus, and, to a lesser extent, high potash and high magnesium. The other criteria (emergence, blooming, number, and weight of corms) indicated the earliest or greatest response from low phosphorus, high potassium, and high magnesium. The effect of nitrogen, however, was variable. Low nitrogen produced the most healthy plants, tallest spikes, most healthy and greatest total number of corms, and greatest total weight. High nitrogen stimulated early emergence and produced the most plants, most spikes, and largest corms. High nitrogen also delayed flowering, as did high phosphorus and low potash, whereas magnesium had little effect on this factor. Differences in other criteria were negligible.

The above comments pertain only to the dry rot disease and to general growth response. Supplementary counts were made of corms affected with *Fusarium* basal rot. The numbers were small and may not be indicative. The most *Fusarium*-diseased corms were in the high nitrogen, low phosphorus, high potash, and high magnesium sets.

1957 experiment.—*Stromatinia gladioli* may attack at any time during the growing season in western Washington. Hence, its attacks may be reflected in decreased plant, flower, and corm production, as well as in observable lesions on corms. Therefore, selected data on growth as well as on disease index are presented, together with statistical evaluation of the most important data (Table 2). Other factors are briefly discussed. (Asterisks are used when results were significant* ($P<0.05$) and highly significant** ($P<0.01$); "ns" means nonsignificant at 0.05 level.)

1) *Plant growth.*—Early emergence (on May 24), total emergence (by June 19), and total number of healthy plants remaining on August 13, were, in general, greatest from corms with a history of low amounts of nitrogen, phosphorus, and magnesium. Low potassium was also better than high potassium, except for total stand on June 19; by that date the high level had produced the most plants in 2 of the 3 varieties. The most healthy plants on August 13 were from corms from low nitrogen, low magnesium, high phosphorus, and high potassium stocks.

TABLE 2. Disease index, weight of corms, and number of corms harvested/corm planted in 1957[a] with different types of previous nutrition

Previous nutrition (NPKMg)[b]	October Sunshine			Spic and Span			Spotlight		
	Disease index[c]	Weight (g)	Corms (no.)	Disease index[c]	Weight (g)	Corms (no.)	Disease index[c]	Weight (g)	Corms (no.)
1111	1.92	28.47	0.71	1.46	23.92	0.57	1.61	24.92	0.72
1112	2.21	31.12	0.80	1.24	21.52	0.50	1.11	23.14	0.52
1211	1.79	25.36	0.64	1.28	19.45	0.47	1.51	24.84	0.60
1212	1.44	21.31	0.53	1.12	16.27	0.43	1.07	13.88	0.43
1121	2.02	29.08	0.69	1.17	18.69	0.53	1.33	20.51	0.59
1122	1.67	25.26	0.67	1.33	20.33	0.58	1.20	24.51	0.57
1221	2.25	30.16	0.73	1.68	27.91	0.64	0.95	14.62	0.44
1222	1.83	28.15	0.67	1.64	20.51	0.57	1.50	26.18	0.66
2111	1.60	29.45	0.59	1.38	22.85	0.56	0.94	14.82	0.44
2112	1.54	25.35	0.67	1.19	21.58	0.54	1.00	20.29	0.44
2211	1.73	26.19	0.61	1.88	30.00	0.73	1.11	22.07	0.49
2212	1.20	23.22	0.47	1.01	20.29	0.46	0.72	14.55	0.35
2121	1.76	28.49	0.60	1.31	20.11	0.50	0.90	18.25	0.40
2122	1.56	23.95	0.57	0.99	17.96	0.40	1.03	19.39	0.52
2221	1.84	26.84	0.71	2.15	34.47	0.78	1.38	29.86	0.56
2222	1.23	22.69	0.44	0.75	9.51	0.34	0.83	13.10	0.34
Average	1.72	26.56	0.63	1.35	21.58	0.54	1.13	20.30	0.50
Error MS	0.10	22.97	0.01	0.25	92.30	0.02	0.12	84.36	0.02

[a] Total planted/treatment: 215 for October Sunshine; 110 for Spic and Span; 95 for Spotlight.
[b] 1 and 2 indicate low and high levels of nutrients in the order N, P, K, and Mg.
[c] Smaller values indicate greatest disease.

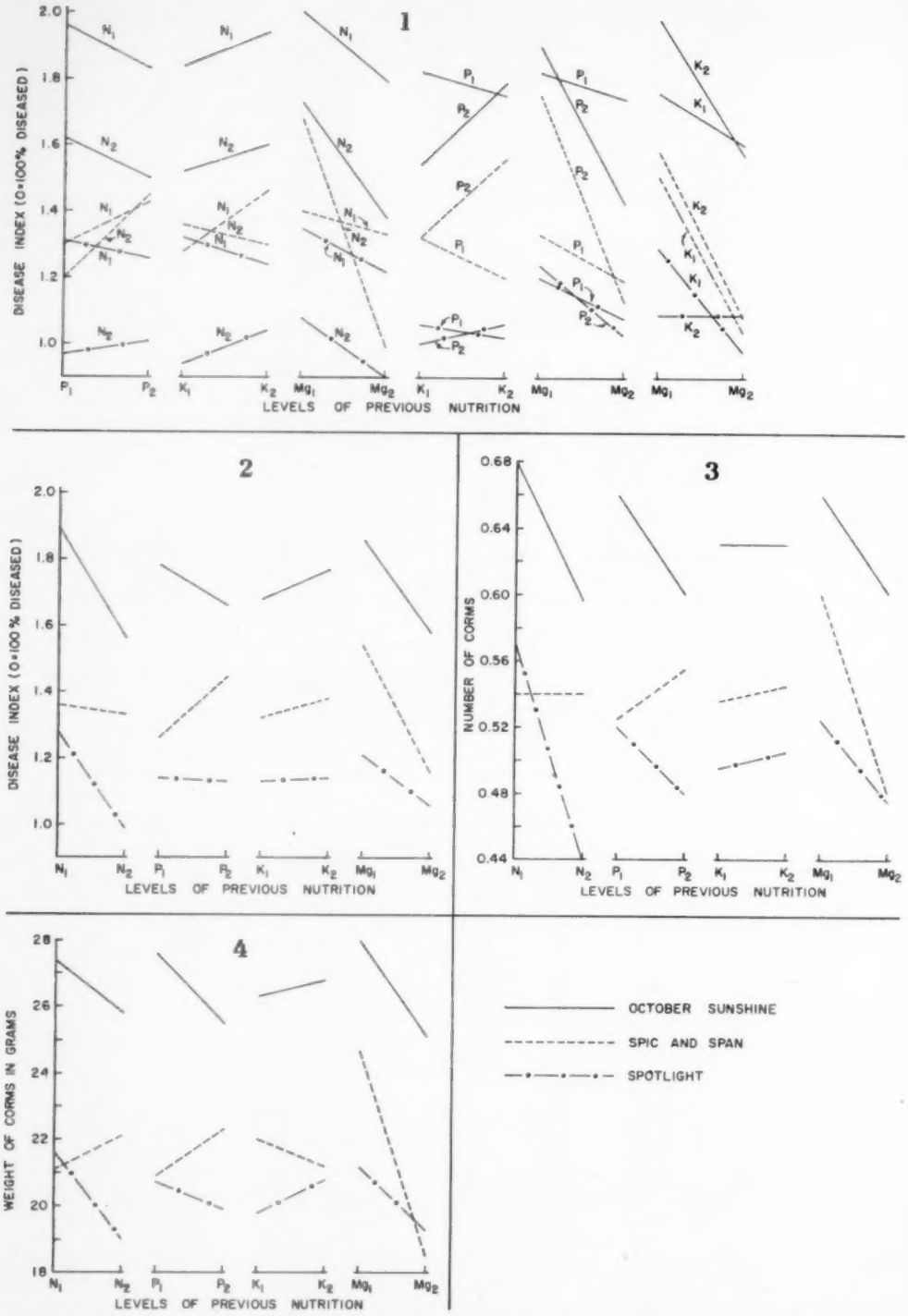

Fig. 1-4. **(1)** Graph of 1st-order interaction of disease index. **(2)** Graph of the disease index/corm planted for the levels of previous nutrition. **(3)** Graph of the number of corms harvested/corm planted for the levels of previous nutrition. **(4)** Graph of the corm weight harvested/corm planted for the levels of previous nutrition.

2) *Flower production.*—Early flower production was slightly stimulated in all 3 varieties by a high-nitrogen history, but total flower production was better from low- than from high-nitrogen sources. Early blooming was also favored by low phosphorus, low potash, and high magnesium. The tallest spikes came from high nitrogen, low magnesium, and (in 2 of 3 varieties) low phosphorus and high potash; the reverse results with phosphorus and potassium in Spotlight, however, indicate that varieties may respond differently.

3) *Disease index.*—The disease index should reflect the weight and number of harvested bulbs, as well as the disease loss. The following discussion pertains only to the disease index, although separate analyses were made of both weight and number of corms. Fig. 2, 3, and 4 show the similarities of index, weight, and number harvested.

a) *Nitrogen.*—In all 3 varieties the low level of nitrogen resulted in the least disease. The differences were significant in October Sunshine** and Spotlight**, and nonsignificant with Spic and Span.

b) *Phosphorus.*—These results were variable and nonsignificant. As phosphorus was increased, disease loss decreased with October Sunshine, increased with Spic and Span, and stayed about the same with Spotlight.

c) *Potash.*—Increasing the potash level slightly decreased the disease with October Sunshine** and Spic and Span**, but had no appreciable effect on Spotlight**.

d) *Magnesium.*—This nutrient had the most effect of all. Increasing it resulted in a great increase in disease for October Sunshine**, Spic and Span**, and Spotlight*.

e) *Interaction.*—Several interactions occurred, but they mostly varied with the variety. A PMg interaction was present in October Sunshine** and Spic and Span*. In both cases an increase in magnesium increased the disease with both low and high levels of phosphorus.

A PK interaction of October Sunshine* appears to have been caused by an improper balance of nutrients, with best results from either low or high levels of both.

In Spic and Span, interactions of NMg** and PMg* were alike and were attributed to the fact that increasing magnesium increased the disease loss much more at high levels of either nitrogen or phosphorus than at low levels.

A KMg* interaction developed in Spotlight; increased magnesium increased disease loss at low levels of potash but not at high levels.

The above-mentioned 1st-order interactions are illustrated in Fig. 1. There were 3 significant 2nd-order interactions; namely, NPK* in October Sunshine, and NPMg** and NKMg* in Spotlight.

DISCUSSION.—Results in 1957 indicated a general correlation between the 3 main factors of harvest weight, number, and disease index of corms. The varieties differed in their response, however; October Sunshine and Spotlight often reacted similarly, and different from Spic and Span. The difference was particularly striking where Spic and Span reacted favorably to an increase in phosphorus and showed either a different or insignificant response to nitrogen increase. Both results were opposite to those of the other 2 varieties. This variation is probably expected, although all 3 varieties have Picardy as 1 parent (2). Because of these indications, it would be quite dangerous to make definite assumptions or recommendations for fertilizer programs for dry rot disease control until tests are made on enough additional varieties to determine the majority response. Likewise, climate, soil type, and other factors undoubtedly affect results.

Magnesium had the most pronounced effect of all the elements tested. An increase in this material drastically decreased the number and weight of corms harvested and increased the amount of disease. Magnesium was also involved in most of the significant interactions. These results do not correspond with those in 1956, which may be attributed to differences in variety or climate or the nonsignificance of the results in that year. Marshall (7, 8) found that magnesium was necessary for growth of the fungus in culture and also that it affected sclerotial development. Therefore, the effect of magnesium in our field tests may be a direct one on the fungus.

Increasing the amount of nitrogen was the next most important factor in increasing loss from disease and decreasing the numbers and weight of harvested corms in 2 of the 3 varieties. Here also, Marshall (8) has shown that the fungus grew best in culture with higher levels of nitrogen, although the differences in the tests were statistically nonsignificant. Therefore, it is possible that the effect of nitrogen was more of increasing susceptibility of the host than of aiding the fungus in our tests. Marshall (7) also showed that an increase of nitrogen increased the size of sclerotia.

A low level of phosphorus generally produced slightly better results than a high level in our tests, although most of the results were not statistically significant. Marshall (8) obtained slightly better growth of the *Stromatinia* in culture with high levels of phosphorus, primarily in combination with high nitrogen. He (7) also reported an effect (unstated) of P on sclerotial development in culture.

Potash had a less striking effect than nitrogen, but, with only 1 exception, the data for all 3 varieties and measurements corresponded. An increased amount of potash slightly increased corm weight and number as well as disease loss. In Marshall's (8) tests, an increase in potassium in culture slightly increased growth of the fungus. In the absence of potassium, however, the fungus grew much better than when either nitrogen or phosphorus was lacking. Marshall (7) found in culture tests that it was more effective than other elements in speeding up sclerotial production by the *Stromatinia*. High rates produced more

sclerotia than did low rates. Hence, its effect may have been more on the fungus than on the host in our field trials.

The interactions (Fig. 1) between nutrients and varieties on disease loss are difficult to evaluate. Although many were significant and some were highly significant, they were not usually the same for all 3 factors or varieties. Increasing magnesium with all 3 other elements increased disease loss. High levels of potash and phosphorus gave somewhat better results than low levels in the presence of low magnesium. Potash and phosphorus needed to be balanced (both high or both low). There was no significant 1st-order relation between nitrogen and P or K. Marshall (8) reported that at low levels of nitrogen, there was no significant increase in growth of the dry rot fungus when either P or K or both were increased. At a high level of nitrogen, however, both P and K had highly significant effects on growth, with the former being the more important.

Although the solutions were balanced for desired high and low ratios of N, P, K, and Mg, the pH as well as levels of Ca, S, and Cl varied according to the types of compounds used. It is entirely possible that these variables played a part in controlling the uptake of elements such as K and Mg, and in inducing resistance themselves. Subsequent studies should consider these possibilities.

The results indicate a strong effect of previous nutrition on infection by the dry rot fungus. McClellan and Stuart (11) and Brewer (1) state that growth of gladiolus during the 1st month depends on the nutrients stored in the corm, but that after the 1st month current nutrition becomes quite important. Since the dry rot fungus can attack at any time during the growing season in western Washington, it is likely that more detailed tests on current nutrition would also show some effect on infection here. It is also possible that the amounts found necessary for disease response would not be as great as for growth response, and results from current nutrition would, therefore, be less marked. In any case, the available data indicate that the cumulative effect of a proper nutrient balance over a period of years could, by promoting disease resistance, play an important part in control of the dry rot disease. This cumulative effect would also very likely occur with other gladiolus diseases as well as diseases of other types of bulbs.

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PHYTOPATHOLOGICAL NOTES

TEMPERATURE CONVERSION OF RUST RESPONSE OF BEAN

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Contribution No. 271 from the Department of Botany and Plant Pathology, The Pennsylvania State University, University Park. Approved for publication November 22, 1960, as Paper No. 2508 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

The first paper of this series (2) reported that during a particular period of colonization by the bean rust fungus (*Uromyces phaseoli* var. *typica* (Reb.) Wint.) 7 days of 32°C induced a locally necrotic response in a host normally fully susceptible. The present paper describes the induction and appearance of this response.

Temperature modification of rust responses is evidently common, but there are few cases reported in which a susceptible response is changed to a necrotic one. Tables 1 and 2 of Syamananda and Dickson (3) illustrate the situation well. Most changes induced by temperature were small, the reaction being altered from 1 susceptibility type to another. In those cases

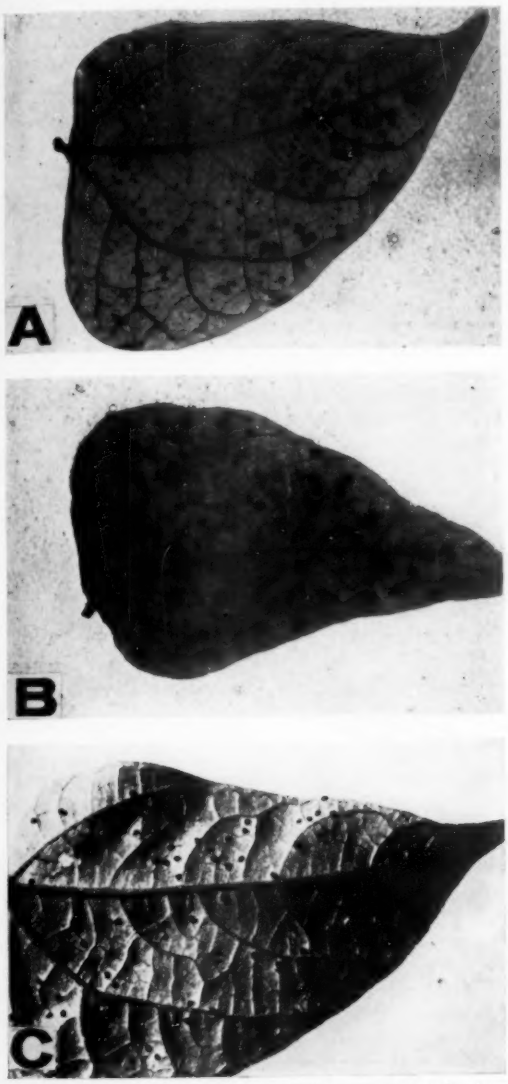

Fig. 1. Responses of Pinto 111 bean to rust infection. **A)** Necrotic response evoked when infections were 96 hours old at time of subjection to 5 days of 32°C. **B)** Necrotic response evoked when infections were 120 hours old at time of subjection to 5 days of 32°C. **C)** Normal response at same time.

involving *Puccinia sorghi* Schw. lines 921 and 925s and inbred corn line Pop 35, however, a cooler than optimal temperature (16°C) could bring about the change from fully susceptible (type 4 pustule) to highly resistant (type 0; necrotic fleck). At 24°C, the "normal" susceptible response occurred. The resistant (type 1) reaction of Pop 35 to line 925s at 28°C suggests an interaction of the type described here, in which high temperature brings about a very large change in response.

The methods of handling and growing plants were completely described in the earlier paper. Special methods (1) used in the present work were the clearing of leaves by gently heating in lactophenol (Amann's medium) to which cotton blue was added and photographing the results on high speed color film from which the figures were made.

The results reported here are from 2 experiments in a series performed to determine the effects of temperature on development of rust on Pinto 111 bean (2). Plants grown for 4 weeks at day-night temperatures of 21-15.5°C, were inoculated with dry spores in a settling tower, placed in a moisture chamber for 24 hours, and then removed to a controlled environment room maintaining day-night temperatures optimal for disease development (27-21°C). After varying periods in this environment, the plants were moved to a room kept at 32°C. Distinct local necroses appeared in 7-10 days on plants receiving certain treatments. If colonization had proceeded beyond a certain stage, heat would not induce the reaction.

In another experiment, plants which had been inoculated 72, 96, 120, and 144 hours previously were subjected to 5 days of 32°C. Infections 96 (Fig. 1-A) and 120 hours old (Fig. 1-B) produced the necrotic response. Seventy-two-hour-old infections were sterilized before sufficient areas were colonized to show the necrosis macroscopically. The infection foci, however, could be seen in transmitted light. Infections older than 120 hours at the time of heat treatment were stopped in development but no local necrosis developed. Actual infection follows a germination-penetration period of at least 6 hours.

The literature does not suggest a simple interpretation of this phenomenon. Previous experiments (2) had made it reasonably certain that the fungus was much more adversely affected by the heat treatment than was the host. Possibly, elevated temperature increased the host's metabolism rate and rendered the host more capable of combating the weakened fungus. This hyperactivity, however, did not show as local necrosis unless colonization had proceeded long enough to give a visible area of affected tissue.

Why infections older than 120 hours did not give the necrotic response is unknown. The phenomenon might be interpreted in terms of establishment of the delicate balance of obligate parasitism. Perhaps, after a certain advanced stage of colonization is reached, the metabolisms of host and parasite had developed adaptively to the point that intense host reactions were not induced by higher temperatures. It is possible also that at that time the parasite had interfered with the host's metabolism to the point that the host was incapable of the hypersensitive response.

On the other hand, one cannot overlook the possibility of a fungus-produced toxin or substance affecting cell-wall permeability, but it is then difficult to explain why such a toxin was not present or active in the infections older than 120 hours.

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BEHAVIOR OF WESTERN X VIRUS IN WELL-ROGUED PEACH AND CHERRY ORCHARDS

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The western X virus, with its various strains and peculiar behavior characteristics, has been investigated in detail by workers throughout the United States and Canada. These investigations have accumulated much data, but a great deal is yet to be learned before all the economic problems associated with the virus can be solved.

The western X virus is only 1 of many viruses which infect stone fruit trees, but it is 1 of the most important. In cherries, the fruits fail to reach a condition of maturity that will permit their marketing as fresh fruits, or they may not mature sufficiently for any purpose whatever. The virus can be lethal to peach trees, and sometimes cherry trees (4), within 2 or 3 years. In both peach and cherry trees, the symptoms are characteristic and prominent (1).

The disease has been sufficiently destructive throughout the western United States that state-wide surveys have been conducted in most of the fruit-growing regions. In Idaho, every infected peach and cherry tree was marked from 1948 through 1951 (3). In conjunction with this program, grower education was emphasized in an effort to get all marked trees removed as soon as possible.

By 1951, the goal of the original Idaho survey had been achieved. Growers had been familiarized with the symptoms of the disease and tree removal was proceeding satisfactorily. The state-wide survey was terminated at the end of the 1951 season; but in 1952, a new kind of survey was undertaken to determine just how effective the recommended tree removal measures were, and how serious a threat to cherry orchards infected peach trees were in the immediate vicinity.

Table 2. Effect of tree removal on spread of the Western X-little cherry virus in individual cherry orchards

Orchard		No. of infected trees					
Variety	No. of trees	1952	1953	1954	1955	1956	1957
Montmorency	111	4	2	1	0	0	0
Montmorency	764	4	1	3	2	2	0
Bing	265	2	1	0	0	0	0
Bing	423	1	0	0	1	0	0

Materials and Methods.—Based on the 1948-51 survey (3), orchards were selected because they contained only a few infected trees during 1951 or because they were interplanted peach and cherry trees.

Selected orchards were mapped and examined on a tree-by-tree basis from 1952-58, with spot checks made in 1958-59. Each time an infected tree was found it was marked with paint and the grower notified of its location. Immediate removal of infected trees was urged.

Results.—The severity of the symptoms in individual cherry trees varied from year to year, but infected trees continued to show symptoms until they were removed. Tree removal generally took place shortly after they were marked in mid-July.

One factor in the annual variation in symptom severity in cherry trees apparently was zinc nutrition; *e.g.*, 1 orchard contained numerous trees that were rated as probably infected in 1952, but after applications of zinc in the following dormant period the questionable cases no longer were discernible.

Fewer infected trees were found in each orchard on each succeeding year in almost all cases (Table 1, 2). The roguing practice, however, produced most rapid decline in numbers of infected trees in the cherry orchards.

In 3 orchards of interplanted peach and sweet cherry trees, infected peach trees were found in each orchard in the early years, but the numbers of such trees declined steadily (Table 3). No infected cherry trees were found.

Discussion and Conclusions.—The failure of the virus to infect cherry trees in orchards interplanted with peach trees, some of which were infected, sug-

Table 1. Annual incidence of the Western X-little cherry disease [a]

Orchard type	No. of trees affected								
	Total	1952	1953	1954	1955	1956	1957	1958	1959
2,669 sweet cherry trees in 13 orchards	42	39	5	2	1	0	1	0	0
160 sour and 113 semisweet cherry trees in 2 orchards	32	12	14	11	3	3	2	0	0
755 peach trees in 8 orchards	23	17	11	6	1	0	1	0	0

[a] Unrogued orchards were not available as controls.

TABLE 3. Effect of tree removal on the spread of the Western X virus in selected orchards of interplanted peach and sweet cherry trees

Orchard		No. of infected trees					
Type	No. of trees	1952	1953	1954	1955	1956	1957
Orchard A							
Peach	150	7	6	0	0	X[a]	X
Cherry	163	0	0	0	0	0	0
Orchard B							
Peach	67	1	1	0	0	0	X
Cherry	63	0	0	0	0	0	0
Orchard C							
Peach	389	4	0	2	X	X	X
Cherry	143	0	0	0	0	0	0

[a] Trees removed.

gests that natural spread of the virus from infected peach trees to cherry trees occurs infrequently, if at all, under Idaho conditions.

The continuing decline in disease incidence in both peach and cherry orchards demonstrated that spread of the virus is slow enough so that diligent tree removal, if practiced early, can eliminate it from infested orchards. Unfavorable environmental conditions during the survey period, however, may have contributed to the rapid decrease in spread. Reduction in symptom severity was noted in association with cool seasons and increased zinc nutrition. Perhaps also, dissemination of the virus was affected by the cyclic nature of vector populations and/or new insecticide programs. Data were not accumulated which would clarify the importance of these factors.

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PLANT-TO-PLANT TRANSFER OF STONE FRUIT RINGSPOT VIRUS IN SQUASH BY POLLINATION

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Technical Paper No. 1380, Oregon Agricultural Experiment Station.

Although the stone fruit ringspot virus frequently spreads into virus-free trees in orchards, the actual means by which this is accomplished has not been reported. Way and Gilmore (8) reported that infected pollen could cause virus-infected seeds in sour cherry, but they did not demonstrate transmission to the mother plant. There are reports (1, 4, 7) that seeds may become infected with other viruses when the flowers are pollinated with pollen from virus-infected plants.

Milbrath and Swenson (6) reported natural spread of the stone fruit ringspot virus in field plots of Buttercup squash (*Cucurbita maxima* Duchesne), but either insect or pollen transmission could have been involved. Das, Milbrath, and Swenson (2) reported that squash plants became infected when stigmas of normal plants were rubbed with pollen-bearing anthers of squash plants infected with stone fruit ringspot virus. Although several squash plants apparently became infected from infected pollen, there was a possibility of mechanical inoculation. In the study here reported, precautions were taken to eliminate virus transmission other than that from infected pollen.

The virus used in these studies has been designated as Oregon RS 31 strain of stone fruit ringspot virus, instead of the controversial names of peach ringspot virus or necrotic ringspot virus (5). After systemic infection through Buttercup squash and then to *Prunus mahaleb* L. seedlings, it will cause typical peach ringspot shock reaction on peach, followed by peach stunt; a ringspot shock reaction on Montmorency sour cherry, followed by sour cherry yellows symptoms; prune dwarf symptoms on Italian prune; and a local necrotic reaction on Shiro-fugen flowering cherry.

Plants of the Buttercup variety of squash were grown in a greenhouse away from any outside source of virus infection. When the plants were in the 8-10-leaf stage, the terminals were removed to force branching. Each plant was limited to 2 branches and these were trained on strings to prevent contact with adjacent plants. The female flowers were tied before opening to prevent chance pollination. Thirty control plants were grown and handled in the same manner, but were not pollinated artificially. Flowers were left open for natural pollination as a further control on chance infection.

Two hundred and four flowers on 97 plants were pollinated with pollen from a squash infected with RS 31, a distinctive strain of the stone fruit ringspot virus. The pollen source plants were grown in another greenhouse to isolate them from the plants to be pollinated. The pollen was collected from the infected plant by suction into a glass vial. Pollinations were made by opening the tied flower, blowing the pollen on the stigma, and retieing the flower closed until it withered.

The pollinations with pollen from infected flowers were made at intervals during a 50-day period, and were completed 21 days before the final records were made. Nine of the 97 plants which were pollinated with infected pollen developed symptoms in the tip

of the plants, typical for those of the RS 31 strain of stone fruit ringspot virus. None of the 30 control plants became infected during the experiment. This experiment confirms earlier observations that squash plants can become infected by pollen from plants infected with the stone fruit ringspot virus (2).

There were 21 flowers pollinated on the 9 plants which became infected. Ten of these flowers developed normal fruit, 8 showed partially developed fruit, and 3 showed little or no development of the ovary. All but 1 of the infected plants developed 1 or 2 normal fruits, 1 plant had a partially developed fruit.

Normally, a mature squash fruit produces 100 or more seeds, indicating 100 or more fertilized ovules in each fruit. Therefore, 2,000 or more ovules could have been involved in the 9 plants which became infected. Likewise, the 187 fruits on the other 88 plants in the experiment would have had a potential of 18,000-19,000 infection sites. Since none of these became infected from pollination with pollen from virus-infected plants, apparently this type of virus infection would be uncommon. Since a cherry fruit develops from a single ovule, demonstration of such transmission from cherry-to-cherry would be difficult. A mature tree, however, produces hundreds of blossoms each year and transmission of the virus by infected pollen to virus-free trees in an orchard could account for the natural spread noted for this virus.

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OAT SMUT RACES OF THE SOUTH ATLANTIC STATES

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Loose smut (*Ustilago avenae*) of oats has been reported from all oat-growing sections of the world (1). In the United States, this disease is most serious in the South Atlantic States with reported annual losses of up to 35% (4). These losses are due to a build-up of a smut population with a broad pathogenic spectrum. The diversity of the smut population was stimulated by the release of oat varieties with widely different germ plasm (6). The diversification of the pathogenic smut population of the southeastern area is substantiated by the susceptibility of varieties that were smut resistant when released. Therefore, a study was initiated in 1958 to survey and identify the smut races of this area. Out of 8 different spore collections from certain states in the South Atlantic region (North Carolina, South Carolina, and Georgia), 5 have consistently conditioned differential reactions on the oat varieties used for differentiating loose-smut races of the Southeastern United States (6). This paper com-

Table 1. Percentage infection[a] of 5 of the southern smut differentials to 5 loose-smut collections under greenhouse conditions

Smut collection	Fulghum	Southland	Atlantic	AB 301	Victorgrain
1	80	90	80	90	0
2	0	95	90	0	0
3	70	5	4	4	0
4	80	70	20	5	0
5	0	0	0	0	95

[a] Percentage infection (an average of 2 tests) was calculated from 4 replications of 20 plants/test. A reaction of less than 10% was considered resistant; 10-30%, intermediate; above 30%, susceptible.

Table 2. Percentage infection[a] of 10 standard differential oat varieties to 5 loose-smut cultures under greenhouse conditions

Oat variety	Smut collection 1	2	3	4	5
Anthony	70	80	80	85	20
Black Diamond	70	75	70	50	3
Victory	35	35	60	25	2
Gothland	0	35	2	0	0
Monarch	0	90	0	0	0
Fulgham	65	0	80	70	0
Black Mesdag	0	0	0	0	0
Camas	0	2	0	0	0
Nicol	0	0	0	0	0
Atlantic	95	90	4	20	4

[a] Percentage infection (an average of 2 tests) was calculated from 4 replications of 20 plants/test. A reaction of less than 10% was considered resistant; 10-30% intermediate; above 30%, susceptible.

pares the pathogenic pattern of these 5 smut collections with those of previously described races.

The smut collections and the method of inoculation were previously reported (5,6). The current tests, however, were conducted in the greenhouse using 5 plants/8-in. pot and 4 pots/treatment. The standard differentials were obtained from the Regional Smut Research Laboratory, Pullman, Wash.; and were used to compare the reactions incited by 5 of the collections with those of previously described races. The reactions incited by these 5 collections on 5 of the southern differentials (6) also were determined under greenhouse conditions.

Data in Tables 1 and 2 indicate that collection 1 is the same as race A14 (3) and collection 2 is the same as A20 (2). Collections 3 and 5 apparently are different from previously described races and have been designated respectively as races A21 and A22. Race A21 is a "Fulghum-type" smut (2,3), whereas A22 exhibits a pathogenic pattern similar to, but not identical with, the "Red Rust Proof" smuts (6,7,8). Collection 4 is somewhat similar to A14, when the data in Table 2 are considered; but causes an intermediate reaction on Atlantic which is susceptible to A14 (3). This fact, coupled with the reaction incited by collection 4 in comparison with collection 1, on the southern differentials, indicates that collection 4 is similar to, but not identical with, A14; therefore, collection 4 has been designated as A14-B.

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THE INFLUENCE OF REDUCING SUBSTANCES ON RESISTANCE TO BACTERIAL WILT IN TOBACCO

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In an investigation of the physiological responses of tobacco plants to infection by *Pseudomonas solanacearum* E. F. Sm., causal agent of bacterial wilt, polyphenol oxidase activity was much greater in infected than in healthy stem tissues (6). Polyphenol oxidase may be involved directly or indirectly in resistance of host plants to pathogenic microorganisms (1) including *P. solanacearum* (5). Henze (2) and other workers have indicated that glutathione and ascorbic acid effectively inhibit polyphenol oxidase activity. To determine whether such inhibition might alter the expression of host resistance, the effect of glutathione and ascorbic acid on resistance in tobacco against bacterial wilt was determined.

Two flue-cured tobacco (*Nicotiana tabacum* L.) varieties, the moderately resistant Coker 139 and the susceptible Bottom Special; and 1 tobacco breeding line, the highly resistant NC 7107, were used in these experiments. Plants were grown in 6-in. pots containing sandy loam soil treated with methyl bromide. Approximately 6 weeks after transplanting, resistant and susceptible plants were treated by pouring 50 ml of water, or $2.7 \times 10^{-3}M$ solutions of glutathione or ascorbic acid over the soil of each pot. Plants were inoculated 48 hours later with *P. solanacearum* by a stem-puncture technique (7). Disease severity was rated using a previously described semilogarithmic index (3). Plants were placed in 1 of the following classes: 1 = no disease; 2 = portion of 1 leaf wilted; 3 = 1 leaf less than completely wilted; 4 = 1 leaf completely wilted; 5 = 0.12-0.25 of the leaves completely wilted; 6 = 0.25-0.5 of the leaves wilted; 7 = 0.5-0.75 of the leaves wilted; 8 = 0.75-0.87 of the leaves wilted; 9 = all leaves wilted except the top 1; 10 = all leaves wilted except a portion of the top 1; 11 = all leaves wilted; 12 = plant dead. Mean class values were converted to a disease index value on a scale of 0-100. Ten plants/treatment were used; each test was repeated 3 times.

Two weeks after inoculation, the control plants of

Fig. 1. The effect of $2.7 \times 10^{-3}M$ glutathione on bacterial wilt development in highly resistant NC 7107 tobacco plants. Plants were treated 48 hours prior to stem puncture inoculation **A)** treated, noninoculated plants; **B)** treated, inoculated plants; and **C)** water-treated, inoculated plants, 7 days after inoculation.

TABLE 1. Disease indices of resistant and susceptible tobacco plants that had been treated with glutathione and selected amino acids 48 hours prior to inoculation with *Pseudomonas solanacearum*

Variety or line	Treatment				
	Water (control)	GSH[a]	Glutamic acid	Glycine	Cysteine
NC 7107 (high resistance)	14[b]	91	85	35	19
C-139 (moderate resistance)	67	93	90	63	43
Bottom Special (susceptible)	89	97	91	63	95

[a] GSH = glutathione.
[b] Represents an average of disease indices for 10 plants. Disease severity rated 7 days after inoculation.

the moderately resistant Coker 139 tobacco plants had an average disease index of 38. In sharp contrast, the disease index of glutathione-treated Coker 139 tobacco plants was 95. Similar effects of glutathione treatment were obtained with the highly resistant NC 7107 (Fig. 1). Disease indices in comparable plants treated with ascorbic acid averaged 81. Each treatment series of susceptible Bottom Special tobacco plants had an average disease index of 95, whether treated with water, glutathione, or ascorbic acid. At the concentrations used, these compounds had no apparent effect on the growth of noninoculated plants. Concentrations of glutathione in a semisynthetic liquid medium (4), equal to those which had been added to the soil in greenhouse tests, had a slight inhibitory effect on the wilt bacterium. Additional *in vitro* studies indicated that ascorbic acid at equivalent levels had no effect on bacterial growth. In attempts to determine whether a particular moiety of glutathione could bring about the same effects as the intact tripeptide, the effect of $2.7 \times 10^{-3}M$ solutions of glutamic acid, glycine, and cysteine on resistance was determined. Only glutamic acid significantly reduced resistance (Table 1). *In vitro* growth studies revealed that glutamic acid stimulated bacterial growth.

Since glutathione and ascorbic acid are excellent organic reducing agents, sodium sulfite, an inorganic reducing agent, also was tested. The average disease indices in sodium-sulfite-treated, highly resistant NC 7107 plants were approximately 7 times as great as those recorded for similar check plants, treated with water. Similar but less striking results were apparent in moderately resistant Coker 139 and susceptible Bottom Special tobacco plants treated with sodium sulfite. This compound had no obvious effects on noninoculated tobacco plants at $2.7 \times 10^{-3}M$. At a $2.7 \times 10^{-3}M$ concentration, sodium sulfite had no effect on the growth of the wilt bacterium in *in vitro* growth studies.

On the basis of these data, it is suggested that the primary effect of glutathione and related compounds is not on the wilt bacterium, but on the metabolism of resistant plants.

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EFFECT OF OXYGEN TENSION ON GROWTH OF APHANOMYCES EUTEICHES

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Aphanomyces euteiches Drechs., incitant of a destructive root rot of canning pea (*Pisum sativum* L.), exhibits semi-aquatic habits characteristic of the group of fungi with which it is taxonomically allied. Infection and disease development are favored by high soil moisture (3, 4). Under such conditions, soil and root environments are deficient in O_2. This study was made to determine the effects *in vitro* of O_2 deprivation on growth of *A. euteiches*.

The concentration of O_2 dissolved in the medium (aqueous phase) was proportional to, and regulated by, the partial pressure of O_2 in the gaseous phase of culture vessels. Gas mixtures were prepared in a 20-liter carboy filled with saturated NaCl solution. As the brine was siphoned off to a reservoir bottle, air or N_2 from a 200 ft^3 tank (99.99% N_2) or both were admitted to the carboy at atmospheric pressure in the proportions needed for the desired O_2 concentration.

The concentrations of O_2 were calculated on the basis that air contains, among other gases, about 20% O_2 and 0.03% CO_2 and that there was .01% O_2 contaminant in the N_2 gas. The influence of the latter 2 low concentrations of gas was considered negligible. The gas mixture was swept through the culture vessel by siphoning the brine back into the carboy and driving the gas through a glass line entering the culture vessel through a 2-hole rubber stopper. A cotton-filled "U" tube between carboy and culture vessel excluded contaminating organisms. The rubber stopper then was sealed with paraffin and aluminum foil.

For visual observations of hyphal growth, discs 13 mm in diameter with the mycelial frontier 0.33 of the distance across the disc were cut from petri dish cultures and placed on 0.75-in. circular cover slips. These were sealed on van Tieghem cells fitted with 2 side arms and fastened to glass microslides. There was a ground glass stopcock in each side arm. Two liters of gas mixture were swept through each cell 2 hours after preparation and again 4 hours later. Then, 3 or 4 hyphal tips were selected for study and all growth originating from these tips in 1 hour was measured with an ocular micrometer. During this period, the rate of elongation was 7.4, 7.4, 7.5, and 7.9 μ per hypha per minute under O_2 tensions of 20, 5, 0.62, and 0.01%, respectively. There was no perceptible change in growth rate during this 1-hour test. Branching was similar in all treatments. Twelve hours after the test began, however, the fungus had grown beyond the edge of the discs at 20 and 5% O_2, but only across 0.67 of the width of discs at 0.62 and 0.01% O_2.

To measure radial growth, 15 ml of potato-dextrose agar in each of several 500 cc Erlenmeyer flasks were seeded with an agar disc of *A. euteiches*. Five liters of gas mixture were swept through each flask before sealing it. Radial extension at 24°C during the first 48 hours was more rapid under 5% O_2 than at lower or higher concentrations (Table 1). Growth in the flasks with 0.01 and 0.67% O_2 ceased at about 72-96 hours. There was little variation between duplicate flasks.

TABLE 1. Radial growth at 24°C of *Aphanomyces euteiches* on potato-dextrose agar in equilibrium with gaseous phases having different O_2 concentrations

% O_2 in gaseous phase	Radius (mm) of colony at indicated hour				
	24	48	72	96	120
20.0	4.5	15.2	28.0	39.0	edge
10.0	4.9	16.0	28.2	38.5	do
5.0	6.5	18.0	29.0	39.0	do
2.5	6.0	16.0	29.0	38.0	do
1.25	5.0	15.5	27.2	37.0	do
0.62	3.2	13.5	24.7	32.5	32.7
0.01	2.0	8.7	20.0	22.5	22.5

TABLE 2. Dry weight of *Aphanomyces euteiches* mycelium formed at room temperature in 160 ml corn-kernel decoction in equilibrium with gaseous phases having different O_2 concentrations

% O_2 in gaseous phase	Dry weight (mg) of mycelium		
	7-day still culture	4-day shake culture	7-day shake culture
20.0	37.1	66.1	88.1
5.0	62.0	79.8	78.9
1.25	32.1	59.4	29.3
0.3	16.0	13.6	15.6
0.01	8.0		9.4

Growth in liquid culture also was studied. Freshly boiled corn kernel decoction was siphoned aseptically into 32-oz prescription bottles until they were filled. The siphon then was reversed. As culture medium flowed out, 800 ml of gas mixture were admitted to each bottle, and 160 ml of medium were left in the bottle. One ml zoospore suspension was added and the stopper was sealed. Cultures were kept at room temperature (about 24°C). Dry weight of the mycelium after 7 days of standing culture or 4 days of shake culture was greater at 5% O_2 than with lower or higher concentrations (Table 2). With 7 days of shake culture, the final growth was greater as the amount of O_2 supplied was increased. There was little growth with 0.3 and 0.01% O_2 under either condition.

These experiments indicate that *A. euteiches* requires O_2 for its growth, but that it grows under very low O_2 tension. Growth in vessels provided with commercial N_2 gas alone apparently occurred at the expense of the small amount of contaminating O_2 in the gas, medium, and rubber stoppers; and ceased when this supply was exhausted. The data also suggest that, at 24°C, 5% O_2 is more favorable for growth than 20% O_2; and that the growth rate is reduced at levels below 1.25%. Very small amounts of CO_2 may have been introduced, but it is doubtful that the results of these experiments were significantly influenced by its presence.

These results are in agreement with the general observation that fungi are obligate aerobes. Often, however, they have relatively low O_2 requirements and sometimes low O_2 optima (1, 2).

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The 9th Annual Meeting and Conference of the Canadian Agricultural Chemicals Association will be held at Mont Tremblant Lodge, Mont Tremblant, Quebec, September 18-20, 1961.

A two-week Conference in Plant Pathology for college teachers of botany and biology is to be held at Purdue University from August 14-25, 1961, with financial sponsorship by the National Science Foundation. The conference is to be administered by the Department of Botany and Plant Pathology of Purdue University with John F. Schafer as Conference Director and R. J. Green, Jr. and E. B. Williams assisting. The purpose of this conference is to bring up-to-date information in the field of plant diseases to some 30 college teachers in the more general botanical and biological fields. Participants will be chosen from college teacher applicants throughout the United States. The aim will be to assist these teachers both in the development of plant pathological phases of their teaching and also in their capacity as counsellors of undergraduate botanical students. It will consist of lectures and discussions of the modern view of six major areas of plant pathology: Nature and significance of plant diseases; Anatomical aspects of plant diseases; Epidemiological relationships of plant diseases; Physiological relationships of pathogenesis; Genetic relationships of pathogens and disease resistance, and Mode of chemical action in control of plant diseases. The instructors will be Paul R. Miller, Crops Protection Research Branch, U.S.D.A.; John H. Owen, Department of Plant Pathology and Plant Breeding, University of Georgia; Paul E. Waggoner, Department of Soils and Climatology, Connecticut Agricultural Experiment Station; Arthur Kelman, North Carolina State College, M. E. Gallegly, West Virginia University; and George L. McNew, Boyce Thompson Institute for Plant Research, Yonkers, New York.

Phytopathologist Robert Goth, who recently received his Ph.D. degree from the University of Minnesota, has joined the Crops Research Division, U.S.D.A., at Beltsville, Md. Dr. Goth will investigate bean and pea diseases.

Phytopathologist Jerry T. Walker began his duties May 1 in a newly created position as Plant Pathologist with the Brooklyn Institute of Arts and Science. Dr. Walker recently received the Ph.D. degree in plant pathology at Ohio State University, Columbus, Ohio.

Phytopathologia Mediterranea is a new journal devoted to pathological problems in the Mediterranean area. The Mediterranean area is regarded not only as a historical unity, but possesses peculiar climatic, ecological and agricultural features which determine the characteristics of its plant disease problems. The publication of this new journal, it is hoped, will contribute to the revival of personal connections among the Mediterranean students of phytopathology and also to a more complete and thorough coordination of plant disease studies in the area. The first issue of the journal appeared in August 1960, containing original papers in various languages. Summaries in English and French are provided. Each volume consists of four issues priced at $4.00. The journal is published under the direction of Prof. G. Goidanich, Instituto di Patalogia Vegetale dell'Universita, Via Filippo Re 8, Bologna, Italy, and Prof. A. Ciccarone, Instituto di Patalogia Vegetale dell'Universita, Via Amendola 165/A, Bari, Italy, to whom contributions may be sent and from whom further information may be obtained.

Plant Pathologist Floyd J. Williams, a recipient of a National Science Foundation Postdoctoral Fellowship, is now at the Department of Plant Pathology, North Carolina State College, Raleigh. Dr. Williams recently received his Ph.D. degree in plant pathology at the Ohio State University, Columbus.

A Conference on Agricultural Meteorology will be held in St. Louis, Mo., November 27-28, in conjunction with the meetings of the American Society of Agronomy.

A Symposium on Weather and Organisms will be presented during the meetings of the American Institute of Biological Sciences, at Purdue University, Lafayette, Ind., August 27-31. The symposium will be jontly sponsored by the AIBS and the American Meteorological Society.

Plant Virologist M. K. Corbett was recently honored by the Florida Chapter of Gamma Sigma Delta, honorary agricultural fraternity. Corbett was selected as the outstanding junior faculty member for his development of the Plant Virus Research Laboratory at the University of Florida and for his outstanding research and teaching activities during the past 5 years.

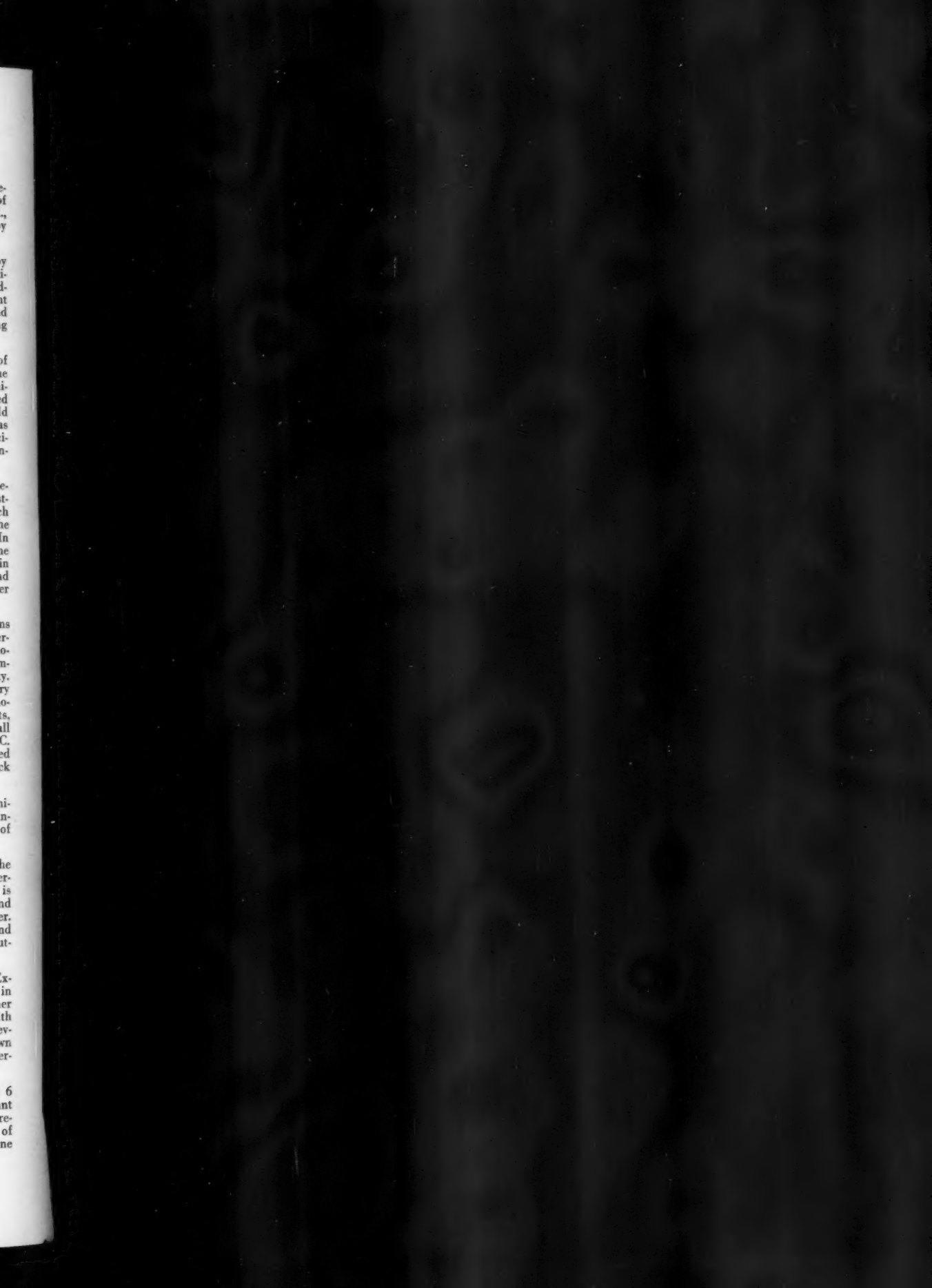
Former Editor-in-Chief E. E. Wilson, Department of Plant Pathology, University of California, Davis, was one of 20 biological scientists invited to participate in a seminar on biological communications. The seminar, sponsored by the American Institute of Biological Sciences, was held in Washington, D. C. June 19 to July 1. Its purpose was to find ways and means for improving the exchange of scientists. All phases of original publication, abstracting, indexing, storage, and retrieval were explored.

Phytopathologist Thomas N. Theis joined the Crops Research Division, Beltsville, Md., on May 1, 1961, as Assistant Chief of the Tobacco and Sugar Crops Research Branch. Dr. Theis was formerly Plant Pathologist at the Federal Experiment Station at Mayaguez, Puerto Rico. In 1953 he was appointed Assistant Officer in Charge of the Mayaguez Station. He has made notable contributions in studies on stem rust of wheat, diseases of forage crops, and a number of tropical crops. He has been a close observer of sugarcane production in Puerto Rico.

The American Society for Microbiology announces plans for a series of annual meetings to be known as "The Interscience Conference on Antimicrobial Agents and Chemotherapy." The first meeting will be held October 31-November 2, 1961, at the Commodore Hotel in New York City. Papers are invited which emphasize the interdisciplinary relationships of the specific aspects of research in chemotherapy and in the nature and action of microbial agents, whether of microbial origin or synthetic. Future plans call for a meeting in Chicago in 1962, and in Washington, D. C. in 1963. Details for the 1961 meeting may be obtained from the American Society for Microbiology, 19875 Mack Avenue, Detroit 36, Michigan.

Phytopathologist Spencer H. Davis, Jr., of Rutgers University, spent part of April in the Republic of Panama investigating tomato diseases for the Nestle Company of Switzerland.

After 41 years of service, J. J. Christensen, Head of the Department of Plant Pathology and Botany of the University of Minnesota, retired June 30. Dr. Christensen is world-renowned for his researches on diseases of cereals and flax. He is equally renowned as an inspirational teacher. Dr. Christensen is the recipient of numerous awards and honors, including the Elvin C. Stakman award for outstanding research in diseases of cereal crops.

Plant Pathologist O. H. Elmer, Kansas Agricultural Experiment Station, retired June 30, 1961, after 31 years in the Department of Botany and Plant Pathology. Dr. Elmer has worked primarily on vegetable and fruit diseases, with emphasis on Irish and sweet potatoes. He developed several outstanding sweet potato varieties, the best known being Laken. He will remain at Kansas as Professor Emeritus and study diseases caused by *Rhizoctonia* sp.

Deaths. Charter Member Frederick V. Rand died June 6 at the age of 78 after a long illness. Dr. Rand was a plant pathologist in the U.S.D.A. for nearly 40 years before retiring in 1947. He is credited with discovering the role of hibernating insects carrying over plant pathogens from one season to the next.